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FILTERABLE VIRUS AND RICKETTSIA DISEASES

BY

EARL BALDWIN McKINLEY

BUREAU OF SCIENCE
MANILA

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247725

Philippine Islands. Bureau of Science
Monograph. no. 27.

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MONOGRAPHS OF THE BUREAU OF SCIENCE
MANILA, PHILIPPINE ISLANDS

WILLIAM H. BROWN, *Editor*
R. C. MCGREGOR, *Associate Editor*
DOROTHY B. O'ROURKE, *Copy Editor*

Monograph 27

FILTERABLE VIRUS AND RICKETTSIA DISEASES

By EARL BALDWIN MCKINLEY

(Actual date of publication, December 31, 1929.)

Reprinted from the Philippine Journal of Science, volume 39, May to August, 1929.

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FOREWORD

Of the diseases affecting man, animals, plants, fowls, fishes, and insects there are over sixty which have been proved to be caused by ultramicroscopic or filterable viruses or are thought to be caused by agents of this nature in the absence of any demonstrable etiologic factor.

Notable reviews* of filterable viruses have been published during the last twenty years among which may be mentioned reviews by Prowazek,(1) Lipschütz,(2) Simon,(3) MacCallum,(4) and Rivers.(5) Most of the available reviews that have been written on this subject have been of an analytical nature of use primarily for the advanced student and investigator whose background of the subject in general is rather broad, and the elementary facts have been omitted as unnecessary for the reader. Such also are the discussions by Wolbach (1912), Doerr (1911), Roux (1903), Loeffler (1911), Bayon (1926), M'Fadyean (1908), Philbert (1924), Kraus (1926), and Twort (1923).

In the following pages the author has brought together a review of the knowledge concerning these various diseases together with a bibliography which it is hoped will be useful for workers and beginning students in this field. While the manner of presenting the material is somewhat similar to text form, it is so presented in order to bring together an inventory of the field and the final chapter will deal in an analytical way with the material which will be presented in the following pages together with contemporary thought on the subject. While the subject matter will emphasize principally the human medical problems involved, there has been included in addition to the diseases of man caused by filterable viruses and rickettsiæ, the diseases of animals, fowls, plants, fishes, and insects which also fall into this category.

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* Since this review was completed Filterable Viruses, edited by T. M. Rivers, published by the Williams & Wilkins Company, Baltimore, has appeared. This notable work contains ten chapters written by ten different men. Each chapter represents a critical essay on separate phases of the subject. The present work aims to be more comprehensive than Filterable Viruses and is presented in a descriptive rather than a critical style.

The study of the filterable viruses and their diseases has within recent years become a highly specialized field in the science of microbiology. At present the approach to the study of these diseases and their viruses is marked definitely on the part of investigators by their background as pathologists and bacteriologists. Occasionally a small beam of light emanating from some slight change in method or technic is focused on particular phases of the subject. Gradually a belief has developed that before any great progress will have been made in unraveling the mysteries attending the study of these agents an entirely new method of approach and attack must be devised. The principles applied in the study of bacteria may in the end be totally inadequate in dealing with the group or groups of filterable viruses. The science of bacteriology has depended largely upon morphology and functional activity of organisms in artificial media for the classification of bacteria, but these methods, for the most part, are not applicable to the study of filterable viruses.

It was the thought in compiling this work that a comprehensive presentation of the available data concerning diseases of man due to filterable viruses along with the similar conditions in animals, fowls, insects, fishes, and plants might serve as a review of the field and possibly aid and stimulate progress in the study of this interesting and important group of diseases.

In the present stage of our knowledge of the filterable virus and rickettsia diseases, we may include four general groups of diseases: (*a*) Those diseases known to be caused by ultramicroscopic and filterable viruses; (*b*) those diseases which in the absence of any demonstrable etiologic agent are thought to be caused by filterable viruses; (*c*) those diseases for which definite bacterial or protozoal forms have been described as etiological agents but which, in many cases, we are unable to accept with finality, or the filterability of the microbe in question has not been definitely settled; and (*d*) the group of diseases known or thought to be caused by rickettsiæ, the ultimate classification of which is undetermined.

Upon the basis of these four groups of diseases this review has been prepared. The author realizes that in many instances the ground is not sure, and the future may determine that many of the diseases included should be classified elsewhere. However, we cannot prophesy what our future knowledge may be in this regard, but we can approximate what is now known and thought concerning the subject.

Apologies then are offered for the vagueness concerning certain diseases and vacillations of contemporary thought regarding many of them which seems to be necessary in view of the limited knowledge concerning these groups of diseases.

The author makes no claim for extensive original work in this field though it is a phase of medical research in which he is most interested. Extensive reading of the work of other investigators has been a great source of pleasure and highly instructive. The author has selected what has appeared to him to be the most important articles dealing with each subject but is well aware that such a selection may involve errors and that others might consider work not cited in these pages as being more important. It is the plan of the author to revise and amplify this entire work at a later date as the development of the subject proceeds. Meanwhile it is offered in its present form as an initial effort to bring together the salient facts pertaining to the subject. It is hoped that this review will be of value and interest.

EARL BALDWIN MCKINLEY.

Bureau of Science, Manila, P. I., *March, 1928.*

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CHAPTER I

INTRODUCTION

DEFINITION

Loeffler in 1898 defined a filterable virus as "the virus of an infectious disease which is so small that it will pass through the pores of a Berkefeld or Chamberland filter." An ultramicroscopic virus is a virus too small to be seen with a microscope. It is most difficult to formulate a definition of a virus which will fit even the meager facts known about viruses to-day and at the same time provide a concept for hypothetical viruses of which practically nothing is known. Undoubtedly we all have a mental concept, or mind picture, of what we consider a virus. We may adhere tenaciously to the belief that all viruses are living agents and are represented by minute bodies of the nature of very small germs or particulate units, or we may consider the vast majority of viruses in this light and recognize the further possibility that viruses may exist in an unorganized state. We may even be willing to admit the possibility that some viruses are nonliving. The facts are that we do not know the exact nature of many so-called viruses, and we do not possess enough accurate information to enable us to make a decisive statement in the matter. We have impressions, ideas, and theories; we have personal convictions based on experience, experiment, logic, and reason; but with regard to many filterable viruses, the great majority in fact, we lack both cultural and morphological data and our information is essentially limited to the relation of these viruses to external physical agents. What then should be our attitude in defining a virus? Future studies in the field of the filterable viruses or protobiology may lead to startling discoveries; and the nature of these minute structures, when it is definitely known, may alter some of our most fundamental theories of life and evolution. Suffice it to say that in the light of our present knowledge we believe the statement is justified that at least one type of virus, and perhaps all types, may be defined as a living particulate agent capable of inducing disease. In this sense we may recognize living particulate agents which are both pathogenic and nonpathogenic and include in this definition bacteria, protozoans, and the filterable viruses. In addition to this group we are further willing to admit the possibility of the existence of a group of unorganized agents which may or may not cause disease. In this group we should place poisons both chemical and animal, toxins, enzymes, etc., and any other hypothetical disease-producing substance or substances which do not form part of our knowledge.

The term "virus" is an old one originally derived from the Latin word meaning poison. Time has modified our understanding of the original translation of this word. Several "virus terms" have been in common usage since the latter half of the nineteenth century, such as "virus animatum," "dehumanized virus," "humanized virus," "virus fixé," and "street virus," with all of which the student is familiar. Some of these terms are little used to-day. Other words have been compounded with the term virus; for example, "viruliferous" [L. virus (poison) and ferre (to bear) hence the definition, "conveying or producing a virus or infective germ"]. Still another term more recently introduced into the literature is "virucidal" or "virucide" [virus (poison) and L. caedere (to kill)] and is defined as "destructive to viruses."

HISTORY

In 1659 and 1675 Kircher, a Jesuit, and van Leeuwenhoek, a Dutch linen draper, invented the simple microscope. These two men were undoubtedly the first to see living cells too small to be seen with the naked eye. From all available records there remains little doubt that these men actually saw bacteria. Whether the imaginations of these two pioneers ever rested upon the possibility of living agents still smaller than those seen with their microscopes or the compound and ultramicroscopes which were to come later will always remain a matter of conjecture. We do know that following the invention of the microscope and the discovery of these minute living beings by Kircher and van Leeuwenhoek the march of events in the science of bacteriology was very slow. It was not until 1762 that Plenciz wrote of the possible relation between bacteria and disease, although the conception of "contagion" had been written of by philosophers for hundreds of years prior to that date. History records the fact that nearly two hundred years elapsed between the discovery of bacteria by Kircher and van Leeuwenhoek and the discovery by Pollender,(6) in 1849, of the anthrax bacillus which was the first bacterium to be definitely proved the etiological agent in an infectious disease.

It is a matter of record, however, that in 1804, forty-five years before Pollender discovered the anthrax bacillus, Zinke had studied the filterable virus disease of rabies in dogs and demonstrated the infectiousness of the saliva, although this disease was not transmitted to rabbits by artificial inoculation until the experiments of Galtier in 1879. The method of immunization perfected by Pasteur during the next few years is one of the landmarks of medical history.

Before bacteriology then was established as a science, investigators were concerned with the study of at least one disease that we now classify with the filterable virus diseases. While bacteriology in general has widened its frontiers to almost undreamed of limits in many directions, comparatively small progress has been made in the study of the filterable viruses. The beginning of the study of filterable viruses is usually given in texts at a later date than that mentioned above. The foundation of this study is usually dated from 1886 when Loeffler and Pfeiffer (7) domonstrated in the lesions of smallpox minute bodies which they thought resembled Protozoa. Shortly after, in 1892, Iwanowski (8) when studying mosaic disease of tobacco found that filtrates of his material remained active for several months. This work is usually considered the corner stone of the study of filterable viruses. The work of Iwanowski was independently confirmed in 1899 by Beijerinck, (9) who advanced the theory of the possible existence of a "contagium vivum fluidum." During the preceding year Frosch and Loeffler (10) demonstrated the filterable nature of the virus of foot-and-mouth disease in cattle. Since that time the study of the filterable viruses has developed in increasing importance and many discoveries have been made which will be described in the body of the text. Briefly some of the more important landmarks in the study of this group of viruses, since the work of Frosch and Loeffler in 1898 on foot-and-mouth disease, set down in chronological order are as follows: In 1903 Negri (11) described the bodies, which now bear his name, in the central nervous system of animals dying from rabies; the same year Borrel (12) demonstrated minute granules, which stain with Leoffler's stain, in sheep pox, the so-called sheep-pox bodies; in 1907 Prowazek (1) demonstrated the so-called trachoma bodies in the epithelial cells of the conjunctiva from cases of this disease, and during that year published a review of filterable virus diseases; Heymann, (13) in 1909, demonstrated "inclusion bodies" in a form of conjunctivitis present at birth known as conjunctivitis neonatorum; Linder (14) during the same year differentiated this disease from gonoblennorrhœa, showing that there might be two diseases, one due to a filterable virus, the other to the gonococcus; during 1908 Landsteiner and Popper(15) succeeded in transferring poliomyelitis to two monkeys from spinal-cord emulsion from human cases which had died of the disease, and demonstrated that the virus was filterable. This work was later confirmed by Flexner and Lewis.(16)

In 1911–12 Rous(17, 18) demonstrated the filterable nature of a sarcoma of chickens, a monumental piece of work which attracted attention again in the work of Gye and Barnard(19) in 1925. In 1913 there were several important contributions to the filterable virus field. Flexner and Noguchi(20) succeeded in cultivating the virus of poliomyelitis; Noguchi and Cohen(21) described the cultivation of minute bodies described by Prowazek as occurring in trachoma; da Rocha Lima(22) demonstrated the filterable nature of the causative agent in verruga Peruviana, or Peruvian warts, thought to represent a later phase of the disease known as Oroya fever. The interpretation of this work, however, may have to be modified in the light of more recent work by Noguchi(23) in 1927, which appears to have established the true etiology of this disease. Finally, during the same year Lipschütz(24) published a complete survey of the entire subject of filterable viruses and presented a list of forty-one diseases affecting man and animals in which the filterable nature of the causative agent was established with more or less certainty. In 1915 and 1917 we find the work of Twort(25) and d'Herelle(26) on the subject of bacteriophage. This phenomenon will be considered in a separate chapter. Until 1918, yellow fever was thought to be due to a filterable virus, and temporarily at least this became doubtful when in that year Noguchi(27) described *Leptospira icteroides* as the cause of this disease. In view of the recent work of Sellards(28) on this subject we are again in doubt regarding the etiology of yellow fever, and for the time being at least we have included it in this review. In 1919 Strauss and Loewe(29) claimed to have demonstrated and cultivated a filterable virus as the cause of epidemic encephalitis. This work, however, has not received confirmation. Levaditi, Harvier, and Nicolau(30) have also described a filterable virus as the cause of this disease, but researches of other investigators as well as our own(31, 32) have shown this virus to be closely identified with the virus of herpes, if indeed it is not the same. During 1920, 1921, and 1922 appeared the work of Olitsky and Gates(33) describing *Bacterium pneumosintes* as the causative agent in epidemic influenza. More recent years have seen the development of the work on rickettsiæ, diverse studies on the various inclusion bodies in attempts to correlate the mass of work on this subject; and lastly the cultivation of the causative agent of trachoma, the transmissibility in series of the experimental conjunctival disease, and the recovery of the microörganism from the experimental lesions by Noguchi(34) in 1927.

ULTRAFILTRATION

Filterable viruses, so we have seen by definition, are so designated because they are small enough to pass through the pores of a Berkefeld or Chamberland filter. The sole requisite, then, for an infectious agent to be so classified, is for it to pass through one of these filters. It must be pointed out in the beginning that filtration is only a matter of gradation. There is no sharp dividing line, and it has been said that "like diffusibility, filtration is only a relative concept." There are several kinds of filters used in the study of filterable viruses, but none is free from objection. In the first place the technical habits of the investigator in the use of these filters is of great importance when results are to be analyzed and evaluated. Pressure, whether suction or force pressure, time of filtration, the dilution of the material to be filtered, its viscosity, the amount of solid matter present, the reaction of the material, the possible variation in size of the virus at the time of filtration—all are important factors which should be taken into consideration. Add to these factors the fact that each filter, whether made of porcelain, diatomaceous earth, collodion, asbestos, plaster of Paris, or any other substance, differs from other filters made of the same materials even though it is only slight in degree—then the margin of safety is even much less, when the investigator attemps to interpret his results.

Aside from the mechanical objections already pointed out there are two factors that should be emphasized; namely, new filters vary individually, and control filters are essential in the interpretation of results. If these two points were always kept in mind the results of filtration experiments would be exceedingly more convincing. For example, the spirochætes that Wolbach(35) passed through a Berkefeld filter are not regarded as filterable viruses because it is believed that due to their flexibility this type of organism may be pulled or sucked through the tortuous pores of the filter. Wherry(36) has shown that an organism that causes a form of pneumonia in guinea pigs and which measures 0.5 to 0.7 micron may pass through a Berkefeld filter; von Esmarch(37) has passed spirilla through a Chamberland filter, and Borrel(38) has succeeded in passing water flagellates through artificial filters. It is possible, however, that cultures of organisms such as have been mentioned above may contain forms, some of which are extremely minute in size, either as a natural condition in the life cycle of the organism or as an abnormally dwarfed form existing as a result of heredity or environment. Recent work on filterable forms of *Bacillus tuberculosis* by Calmette and Valtis,(39) Mellon and Jost,(40) Fabry,(41) Potter,(42) and others, recent investigations on filterable forms of *Bacillus pestis* by Burnet,(43) on filterable yeasts by Lewis,(44) and the discovery by Noguchi(45) of a filter-passing virus obtained from *Dermacentor andersoni,* all lend support to this concept. Undoubtedly the gradations brought about by the filters now available are not fine enough and reports of filterable forms of bacteria which have never heretofore been classified with the filterable viruses, give rise to many forms of speculation. Encouragement may be found, however, in the recent work of Kramer(46) on bacterial filters and of Zinsser and Fei-Fang Tang(47) in their studies on ultrafiltration. Kramer has shown that by preparing a filter of calcium carbonate and magnesium oxide of positive electrical charge, bacteria, viruses, and colloids used in his tests may be withheld, though these agents readily pass through filters made of siliceous material carrying a negative charge. Zinsser and Fei-Fang Tang have attempted to arrive at the relative size of several different substances by filtration at a known P_H through graded filters prepared of collodion. The results of these experiments show an order of magnitude of the various substances tested as follows: Crystallized egg albumen; crystallized serum albumin; trypsin; collargol; casein; bacteriophage, Rous sacroma, and herpes virus; and lastly arsenic trisulphide. These results, as pointed out by Zinsser and Fei-Fang Tang, do not agree with the work of Levaditi and Nicolau(48) and Levaditi, Nicolau, and Galloway(49) in that these investigators found that the virus of foot-and-mouth disease was filterable through membranes that held back trypsin. On the other hand the work of Zinsser and Fei-Fang Tang agrees favorably with the measurements made by Olistky and Boëz(50) for the virus of foot-and-mounth disease.

Our own observations(51) have been that well-controlled experiments with collodion-membrane filtration experiments are very difficult. For example, particles of bacteriophage are for the most part absorbed onto the membrane though it is possible to recover the active principle by successive feedings of the filtrate with the susceptible organism, thus showing that some of the bacteriophage passed the filter although a substance such as hæmoglobin was withheld.

The size of these minute viruses is important without doubt, and at present there is no way in which to arrive at their approximate size except through comparison with other known substances. Far more important, however, is their nature, their life cycle, the methods of cultivating them, and the study of their functional activities. These are all problems for the future, since for most of the filterable viruses none of these things are known. Rawlins,(52) in writing recently of research on viruses causing plant diseases, calls attention to the work of Mines(53) who has shown that the addition of certain protective proteins to colloidal gold may cause the latter to exhibit properties characteristic of proteins. In this respect Rawlins suggests that it is possible that protective colloids and other factors in the complex plant extract may so modify the properties of a virus as to give an erroneous impression of its real nature. Zinsser and Fei-Fang Tang clearly recognize this possibility in their experiments. Rawlins in his view of the field of virus diseases of plants suggests several lines of study; such as, selective absorption, treatment with microöganisms, precipitation, cataphoresis, centrifugation, sedimentation, and dialysis such as have been employed by Sherman, Caldwell, and Adams(54) in their attempt to isolate enzymes. Some of these methods have already been used in the study of filterable viruses causing human diseases though the possibilities have by no means been exhausted. Inactivation of viruses by means of colloids of known composition and reactivation by methods similar to that used by Johnson-Blohm(55) in reactivating rennet is also suggested by Rawlins.

In the end we inevitably return to our conception of filtration which is practically our sole criterion in the classification of these agents. Contrary to general thought Bronfenbrenner and Muckenfuss(56) have recently shown that filters become more permeable the longer they are employed in a given operation. Filtering a strain of bacteriophage and its susceptible organism over long periods of time these authors demonstrated that while in the beginning the active principle passed the filter in a certain concentration, free from bacteria, after prolonged filtration the susceptible microbe itself passed the barrier. More recently there have been several attempts to employ animal membranes in vivo in filtration experiments. In our laboratory we have attempted filtration experiments with a strain of bacteriophage through the normal barriers of the central nervous system.(57) The bacteriophage was injected into rabbits intraspinously and recovered from the blood stream of the animal at a later period. Bacteriophage introduced into the animal intravenously could not be demonstrated in the spinal fluid. Le Fèvre de Arric(58) has shown that certain dyes (such as, methylene blue, trypan blue, and neutral red) and certain drugs (such as, potassium iodide and urotropin) exert a favorable influence upon the fixation by the central nervous system of certain neurotropic viruses, such as the virus of herpes and of rabies, presumably acting upon the vascular endothelium of the meninges. Grasset(59) injected gravid rabbits and guinea pigs with a filtrate of colon bacillus bacteriophage and demonstrated the presence of the bacteriophage in the maternal blood though it was absent in the fœtal blood. Evidently bacteriophage does not traverse the placenta and differs from antitoxin in this respect though toxins and anatoxins are also withheld.

THEORIES CONCERNING THE NATURE OF FILTERABLE VIRUSES

When considering the theories concerning the nature of the filterable viruses one of the first questions that arises is whether these agents are animate or inanimate, or whether some of them are living substance and others nonliving. At present it is impossible to make any statement concerning this question that will apply to the entire group of viruses. Doubtless there are few who would question the living nature of the virus of rabies, of poliomyelitis, of herpes, of hog cholera, of rinderpest, of smallpox, and a host of others, but there are many who have questioned, for example, the living nature of the bacteriophage.

Life (L. vita) is loosely defined as an aggregate of vital phenomena; a certain peculiar stimulated condition of organized matter; that obscure principle whereby organized beings are peculiarly endowed with certain powers and functions not associated with inorganic matter. One of these functions, and perhaps the most fundamental of all, is reproduction. For if there were no reproduction life would cease to exist after a time. Closely following in importance the power of reproduction is adaptation. Living things have the power in general of adapting themselves to their environment. If they did not possess this power they would die. Still another function of living beings is respiration. Even bacteria respire, and their respiration can be measured quantitatively by the methods described by Novy, Rhoem, and Soule,(60) by Bronfenbrenner,(61) and by McKinley and Coulter.(62) These are all fundamental considerations, and while there are many others, such as the assimilation of food, the power of locomotion, sensitiveness to external agents—these are the most important. There can be no question that most of the filterable viruses possess the power of multiplication, and no further comment is necessary in this regard. Upon this point has hinged our conception of the bacteriophage for the past ten years. There are still many who will admit that increase in number of bacteriophage particles takes place under certain conditions but who do not recognize its living nature.(63) Both bacteriophage and herpes virus are susceptible to the destructive action of ultra-violet rays, yet this criterion is not sufficient to determine the living nature of these agents since enzymes are also destroyed by this form of energy.(64)

Filterable viruses possess the ability to adapt themselves to their environment, otherwise they would cease to exist. To be sure, like all living things, they are frequently modified in the process of undergoing adaptation; for example, the modification of rabies street virus when passed through rabbits. That the filterable viruses assimilate various elements for the purpose of surviving and reproducing their kind is indicated in the few successful experiments on cultivation in artificial media; for example, the virus of poliomyelitis. Furthermore, it is well known that this group of agents is susceptible to external changes brought about by chemicals and changes in temperature, although in general they appear to be more resistant than ordinary bacteria in this regard.

The state of being of the filterable viruses meets fully our definition of life. They are beyond peradventure endowed with certain powers and functions not associated with inorganic matter. With the power of reproduction or multiplication, the function of adaptation, susceptibility to physical and chemical agents, ability to assimilate food in artificial culture media, can there be doubt that they are living beings? To be sure it has not been demonstrated that filterable viruses respire, though this may be due to the lack of a suitable method fine enough in its technic to permit demonstration of what may be an extremely small interchange of oxygen and carbon dioxide.

It was first suggested by Beijerinck(9) in his study of the mosaic disease of tabacco that a possible "contagium fluidum vivum" might exist. Simon(3) has suggested another term in his "contagium inanimatum." These two concepts are diametrically opposed to each other as regards the living nature of the "contagium," but both suggest in their meaning that the filterable virus may exist in a chemical form. There is no direct evidence to support this concept, though the theory is of great interest.

A concept that is quite wide spread is that the filterable viruses exist as filterable or ultramicroscopic forms of bacteria and perhaps in some cases as protozoans. The physicist regards them as particulate beings that have up to the present been undemonstrable because of the lack of suitable optical instruments with which to see or photograph them. The particulate nature of a few viruses has been demonstrated, especially those that have undergone artificial cultivation. The susceptibility of herpes virus to ultra-violet light is also suggestive of this, while in the case of the bacteriophage actual partition has been accomplished by d'Herelle,(26) by Bronfenbrenner and Korf,(65) and in our own experiments.(63) It has been suggested by many investigators that the filterable viruses are more resistant to external agents than bacteria because of absorption of the filterable agent upon protein aggregates or vice versa, thus introducing the concept of protective colloids.

Opinion is divided regarding the significance of the so-called cell inclusion bodies, such as, trachoma bodies, sheep-pox bodies, the inclusions of herpes, Negri bodies of rabies, vaccine bodies, intranuclear inclusions in visceral disease described by von Glahn and Pappenheimer,(66) the cellular inclusions in the salivary glands of guinea pigs, Kurloff bodies in guinea pigs, and others. It is quite evident that these bodies for the most part are uniformly present and associated with the respective virus disease. Prowazek in 1907 suggested the name "chlamydozoa" for this group of "cell inclusion bodies," a term derived from the Greek meaning "cloak" and "animal" and defined as a protozoan consisting of a cell surrounded by material secreted by the invaded cell. Reference has already been made to the theory that Negri bodies are in reality protozoan in nature. Lipschütz has suggested the term "strongyloplasma" for this group of bodies, a term also derived from the Greek, meaning "round" and "to mold," hence the definition "to mold round." One school of thought considers these bodies as representing the virus proper, either singly or in groups, while another believes them to be simply the reaction products of the cell massed together and capable of taking stains by which they are demonstrated. The inclusion bodies will be considered in detail in a chapter devoted to this subject.

CLASSIFICATION OF FILTERABLE VIRUS DISEASES

The filterable virus diseases represent one of the most difficult groups of diseases to classify. This is partly due to the fact that new developments in their study necessitate frequent modification, but chiefly because of our lack of exact knowledge concerning many of them. They cannot be classified according to the pathologic changes they produce because in many instances the pathology is unknown; for example, in dengue fever. They cannot be classified according to bacteriologic or protozoan criteria for too little is known of their nature. They cannot be grouped according to transmission because the method of transmission in many instances is doubtful or unknown. They cannot be classified from the cytological point of view for, as Cowdry (67) has stated, "The inclusions themselves, occurring as they do not only in man and many vertebrates, but also in certain insects and plants, are characterized by great diversity. For this reason generalizations are difficult to make, and are often stultified by the number of qualifications and exceptions which must be noted." Further, Rivers (5) in an abstract of his review on filterable viruses states: "In regard to the filterable viruses, it can be said that they exhibit, when compared one with another, a diversity of characteristics equal to, if not greater than, that exhibited by ordinary bacteria and other known forms of life."

In 1912, Wolbach (68) published a chart of the diseases presumably caused by filterable viruses. In this tabulation he attempted to set down the transmission as well as the occurrence of the disease, but in many instances the transmission was not known to be either direct or indirect. While Rivers (5) does not offer his table of filterable viruses as a classification, he has attempted to correlate the known facts in the grouping of filterable viruses diseases, and his table is exceedingly interesting.

Probably the most general classification of this group of diseases can best be made upon the basis of host susceptibility. This factor is quite generally known, and in grouping the diseases according to their occurrence the finer known characteristics of the viruses may be taken into account and upon these points the place of each particular disease in the grouping may be determined. Such a classification follows:

TABLE 1.—*Classification of filterable virus and rickettsia diseases according to host susceptibility.*

I. VIRUS DISEASES OF MAN.

	Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible animals.	Immunity.	Mortality.
A	Variola	Direct	9–15 days; average 12 days.	General	Filterable	Present	Skin	Man, rabbits, monkeys, cattle, rats, camels, guinea pigs.	One attack confers immunity.	Up to 25 per cent.
	Varioloid	do.	4–13 days	do.	do.	do.	do.	do.	do.	Nil.
	Vaccinia	do.	3 days	do.	do.	do.	do.	do.	do.	Do.
	Paravaccinia	do.	do.	Austria	do.	do.	do.	Man	do.	1–2 per cent.
	Alastrim	do.	3–12 days	Africa; West Indies; South America.	do.	do.	do.	do.	do.	Nil.
B	Varicella	do.	14–21 days	General	No report	do.	do.	do.	do.	Nil; fatalities due to complications.
	Herpes zoster	Unknown	Unknown	do.	do.	do.	Skin, nerves, ganglia.	do.	Of very short duration if any.	Nil.
C	Rubeola	Direct	11–14 days	do.	Filterable	Absent	Skin	Man, and monkeys experimental.	One attack confers immunity.	Nil; fatalities due to complications.
	Rubella	do.	14–21 days	do.	No report	do.	do.	Man	do.	Nil.
D	Verruca	Probably direct and indirect.	4 weeks to 6 months (experimental).	do.	Filterable	Present	do.	Man, dogs	None	Do.
	Molluscum contagiosum.	do.	14–25 days (experimental).	do.	do.	do.	do.	Man	do.	Do.

E	Trachoma[a]	Direct	2-4 weeks (experimental in monkeys).	China; Japan; Egypt; Russia; United States.	Filterable(?)	do.	Conjunctivæ.	Man, and monkeys experimental.		Do.
	Inclusion blennorrhœa.	do.				Present	do.	Man		Do.
F	Herpes simplex.	Unknown		General	Filterable	do.	Skin	Man, rabbits, guinea pigs, rats.	Little if any	Do.
	Epidemic encephalitis.	Probably direct.	Few hours to few days.	do.	Probably filterable.	Present (??)	C. N. S.	Man (chiefly adults).	One attack confers immunity.	30–70 per cent.
	Epidemic poliomyelitis.	do.	1–3 days up to 14 or 18 days.	do.	Filterable	Absent	do.	Man (chiefly children); monkeys experimental.	do.	5–30 per cent.
	Rabies	Direct	2–8 weeks up to 1 or 2 years.	do.	do.	Present	do.	Man and various animals.	Immunity conferred by vaccination.	Very high unless treated.
G	Common colds.	do.	Few hours to few days.	do.	Probably filterable.	Absent	Respiratory tract.	Man	Little if any	Nil.
	Influenza[b]	do.	do.	do.	do.	do.	do.	do.	Definite but probably temporary.	Nil; fatalities due to complications.
H	Dengue fever[c]	Indirect; direct experimental.	3–6 days, variable.	Southern United States; Tropics.	Filterable	do.	Skin, throat	do.	Temporary immunity.	Nil.
	Yellow fever[d]	do.	4–13 days; average 4 – 5 days.	South America; Africa.	do.	do.	Skin, liver, kidneys.	Man, monkeys experimental.	One attack confers immunity.	30–80 per cent; 7–10 per cent in natives.

[a] Bacterial (Noguchi).

[b] Bacterial (Olitsky and Gates).

[c] Insect borne.

[d] Insect borne; spirochætal (Noguchi).

TABLE 1.—*Classification of filterable virus and rickettsia diseases according to host susceptibility*—Continued.

I. VIRUS DISEASES OF MAN—Continued.

Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible animals.	Immunity.	Mortality.
H ... Pappataci fever.[c]	Indirect	3-5 days	India; Egypt; South America; Southern United States; Mediterranean.	Filterable	Absent	Nil	Man	One attack confers immunity.	Nil.
I ... Typhus fever[e]	do.	4-14 days; average 12 days.	Europe; Asia; Africa; America; Russia; Mexico.	Not filterable.	do.	Blood vessels.	Man, monkeys, guinea pigs experimental.	do.	Very high.
Trench fever[e]	do.	5-30 days; average 14-30 days.	Prevalent during World War.	Filterable(??)	do.	do.	Man	Temporary	Nil.
Rocky Mountain spotted fever.[e]	do.	3 days experimental.	Western states of the United States.	Not filterable.	do.	Blood vessels, spleen, liver, lungs.	Man, monkeys, rabbits, guinea pigs experimental.	One attack confers immunity.	Very high.
Tsutsugamushi fever.[e]	do.	4-12 days; average 4-7 days.	Japan; Formosa	Filterable	do.	Spleen, liver, pancreas, thyroid.	Man and monkeys experimental.	do.	10-60 per cent.
J ... Scarlet fever[f]	Direct and indirect.	2-6 days	General	Doubtful	Present (??)	Skin, throat	Man	do.	Under 5 years 20-30 per cent.

J... Oroya fever; verruga Peruviana.[a]	Probably direct.	About three weeks.	Peru	do.	Absent	Skin, blood, liver, spleen, endothelium.	Man, monkeys experimental. Rabbits? Dogs?	do.	10–40 per cent.
Epidemic parotitis.[g]	Direct	3–25 days; average 14–21 days.	General	Filterable	do.	Salivary glands.	Man, cats experimental.	One attack confers immunity.	Nil.
Foot-and-mouth disease.	do.	2–7 days	do.	do.	No report in man.	Counterpart of the disease in animals.	See diseases of animals.	Temporary immunity.	Do.

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[a] Bacterial (Noguchi).
[c] Insect borne.
[e] Insect-borne *Rickettsia*.
[f] Bacterial.
[g] Spirochætal (Kermorgant).

II. VIRUS DISEASES OF ANIMALS.

Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible animals.	Immunity.	Mortality.
K... Cowpox	Direct and indirect.	4–7 days	General	Filterable	Present	Skin	Cattle, horses, buffalos, camels, carabaos, hogs, sheep, rabbits, fowls.	One attack confers immunity.	Low.

TABLE 1.—*Classification of filterable virus and rickettsia diseases according to host susceptibility*—Continued.

II. VIRUS DISEASES OF ANIMALS—Continued.

	Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible animals.	Immunity.	Mortality.
K	Sheep pox	Direct	6–8 days	Southeastern Europe; France.	Filterable	Present	Skin, viscera.	Merino sheep most susceptible; coarse-wooled sheep less so; varies with breeds	One attack confers immunity.	2–50 per cent.
	Horsepox	do	5–8 days	England; France; Germany.	Probably filterable.	Present (??)	Skin, mucous membranes.	Horses, cattle, man.	do	Low.
	Goat pox	do	6–8 days	Norway; Italy; Spain; France; Germany; Algeria.	do	No report	do	Goats	Severe attack confers immunity.	Do.
	Swine pox	do	4 days	Roumania; Hungary; Morocco.	do	do	do	Swine, cattle, goats, man.	One attack confers immunity.	Frequently high.
L	Rabies	do	2–8 weeks; 2 years recorded.	General	Filterable	Present	C. N. S.	Dogs, cats, man, cattle, swine, goats, carnivorous wild animals.	Conferred by vaccination.	Usually very high; varies with many factors.
	Infectious bulbar paralysis.	Probably direct.	20 hours to 10 days; 12–26 hours (experimental).	Hungary; Brazil; Russia; Germany.	do	No report	C. N. S.; local.	Dogs, cats, cattle, rabbits, guinea pigs, rats, and mice.	Unknown	Practically 100 per cent.

L.	Borna disease	do	28–36 days in rabbits (experimental).	Germany; Hungary; Belgium; England; Russia; United States.	do	Present	C. N. S.	Horses, sheep, rabbits, mice, guinea pigs, monkeys, fowls.	Little if any	75–90 per cent.
	Distemper	Direct	3–4 days (experimental); may be 2 weeks in natural infection.	General	do	do	C. N. S.; pericardium, heart, muscle, respiratory tract.	Dogs, cats, horses (??)	One attack confers immunity.	40–90 per cent; average 50 per cent.
	Guinea pig paralysis.	do	9–23 days	Europe	do	No report	C. N. S.	Guinea pigs		100 per cent.
M.	Nairobi disease of sheep.[c]	Indirect	5–15 days; average 9 days.	British East Africa.	do	Absent	Stomach, intestine, liver, spleen, gall bladder, heart, lymph glands.	Sheep, goats	One attack confers immunity.	30–71 per cent.
	African horse sickness.[c]	do	6–7 days	South Africa; Abyssinia; East Africa; Arabia.	do	Present	Tissues, viscera.	Horses, mules	One attack confers partial immunity.	35 per cent.
	Catarrhal fever of sheep.[c]	do	4 days	South Africa	do	Absent	Subcutis, spleen, anæmia.	Sheep	One attack confers immunity.	40 per cent.
	Heartwater.[e]	do	14 days	South Africa; Angola; Belgian Congo.	Not filterable.	do	Pericardium, spleen.	Sheep, goats, cattle.	do	Over 50 per cent.

[c] Insect borne.

[e] Insect-borne ***Rickettsia.***

TABLE 1.—*Classification of filterable virus and rickettsia diseases according to host susceptibility*—Continued.

II. VIRUS DISEASES OF ANIMALS—Continued.

Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible animals.	Immunity.	Mortality.
Foot-and-mouth disease.	Direct	2–7 days	General	Filterable	Present	Mucous and serous membranes, heart muscle, skin, etc.	Cattle, sheep, hogs, goats, guinea pigs, man, etc.	One attack confers immunity.	40–70 per cent.
Infectious pustular stomatitis.	do	8 days	do	do	do	Mucous surface of mouth nose, eyes, throat, skin.	Horses, cattle, sheep, hogs, man, fowls.	do	Nil.
Rinderpest	do	24 hours to 24 days; average 3–9 days.	Philippines; India; Africa.	do	No report	Mucous membranes, alimentary tract, glands, organs.	Cattle, carabaos, goats, sheep, hogs, boars.	do	50–75 per cent.
Hog cholera	do	4–18 days; average 8–10 days.	General	do	Present	Intestines, lungs, heart, glands.	Swine, guinea pigs.	do	Variable; 5–90 per cent.

Equine infectious anæmia.	Direct, indirect (?)	5–30 days (experimental); average 17 days.	do.	do.	No report.	Blood, spleen, intestine.	Horses, mules, donkeys.	Little if any.	30–70 per cent.
Pleuropneumonia in cattle.[a]	Direct.	12–16 days; 6–7 days (experimental).	Russia; Spain; Africa; Australia; Asia.	do.	Absent.	Lungs, subcutis, pleura, pericardium, peritoneum.	Cattle, buffaloes, reindeer, camels, yak, bison.	One attack confers immunity.	Very high.
Equine influenza.	Direct, indirect (?)	4–6 days.	General.	do.	No report.	Mucous membranes, lungs, C. N. S.	Horses.	do.	.5–4 per cent; fatalities usually due to complications.
Vesicular stomatitis in cattle.	Direct.	36–72 hours (experimental). In natural infection 4–6 days.	South Africa; Italy; France; England; United States.	do.	do.	Skin, mucous membranes, tongue.	Horses, cattle, guinea pigs, swine.	do.	Nil.
Agalactia contagiosa.[h]	do.	Few hours to few days.	Algeria.	do.	Absent.	Mastitis arthritis.	Goats, sheep.	do.	Low.
Ephemeral fever.	Probably direct.	2–3 days.	Transvaal; South Africa.	do.	No report.	Conjunctivæ, circulatory system.	Cattle, horses.	do.	Do.
Myxomatosis of rabbits.	do.		Montevideo; Sao Paolo.	Probably filterable.	do.	Conjunctivæ, rectum, urethra, subcutis.	Rabbits.		Variable.

[a] Bacterial (Noguchi).

[h] Bacterial (Bridré).

TABLE 1.—*Classification of filterable virus and rickettsia diseases according to host susceptibility*—Continued.

II. VIRUS DISEASES OF ANIMALS—Continued.

Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible animals.	Immunity.	Mortality.
Virus III infection.	Probably direct.		United States	Filterable	Present	Skin, testicle, cornea.	Rabbits	Evidence of active immunity.	
Epizoötic of guinea pigs.	do.	2–5 days	England	Probably filterable.	No report	Spleen, lung, liver, adrenals.	Rabbits (?), guinea pigs, rats (?)		High.
Novy's rat disease.	do.		United States	Filterable	do.		Rats		Practically 100 per cent.
Noguchi's virus disease.	Indirect and direct (experimental).		do.	do.	do.	Spleen	Guinea pigs, monkeys.		
Anæmia of rats.[f]	Probably direct.	2–5 days (experimental).	Hamburg; China; Italy; United States.	Doubtful	do.	Anæmia	Rats		

III. VIRUS DISEASES OF FOWLS.

Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible fowls.	Immunity.	Mortality.
N -- Fowl pox and avian diphtheria.[f]	Direct	6–12 days	General; Italy; Tunis; Cuba.	Filterable	Present	Skin, comb, wattles, mucous membranes.	Chickens, turkeys, pheasants, peacocks, pigeons, water fowls.	One attack confers immunity.	Low; 40–50 per cent.

O	Spindle-celled sarcoma No. I (Rous).	No report	Variable	United States	do	Absent	Tumor growth.	Chickens		High.
	Osteochondro sarcoma No. VII (Rous).	do	do	do	do	do	do	do		Do.
	Spindle-celled sarcoma No. XVIII (Rous).	do	do	do	do	do	do	do		
	Fowl pest	Direct	2–5 days	General	do	Present	Tissues, organs.	Various fowls	One attack confers immunity.	60–100 per cent.
	Chicken paralysis.	Probably direct.	Unknown	United States; Canada.	Probably filterable.	Absent	C. N. S., peripheral nerves, viscera.	Chickens	Natural immunity exists.	High.
	Philippine fowl disease.	do	4–6 days	Philippine Islands.	Filterable	No report	Alimentary tract.	Chickens, ducks, geese, pigeons.	do	Do.
	Leukæmia of chickens.	do	1–2 months	Denmark; Germany.	do	do	Liver, spleen, bones, lymph glands.	Chickens	do	Practically 100 per cent.

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[f] Bacterial.

TABLE 1.—*Classification of filterable virus and rickettsia diseases according to host susceptibility*—Continued.

IV. VIRUS DISEASES OF INSECTS.

Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible insects.	Immunity.	Mortality.
Sacbrood disease of bees.	Probably direct.	5-6 days.	Europe; Australia; United States.	Filterable.	Absent.	General.	Bees (larvæ)	Adult bees and pupæ possess high resistance.	Very high.
P -- Wilt disease of gypsy moth and European nun moth.	do.	2-25 days (experimental).	Europe; United States.	do.	Present.	Fat-cells, tracheal matrix, blood cells, hypodermal cells.	Gypsy-moth and European nun-moth caterpillar.	Genetic immunity probable; some evidence of active immunity.	Do.
P -- Jaundice of silkworms.	do.		Japan; Europe.	Probably filterable.	do.		Silkworms.		High.
Nuclear disease of caterpillar of white butterfly.	do.			do.	do.		Caterpillar of large cabbage white butterfly.		

V. VIRUS DISEASES OF FISHES.

Disease.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible fishes.	Immunity.	Mortality.
Epithelioma of *Barbus*.	Probably direct.		Germany.	No report.	Present.	Epithelium of lip.	*Barbus*.		
Carp pox.	do.		Germany; Austria.	do.	do.	Skin, kidney (?) liver (?) spleen (?)	Carp, other fishes.		

Lymphocystic disease.	do	9 days (experimental); 2½ months.	England; Germany; Austria.	do	do	Skin	Bass, flounders, sargus, sole, crab.		

VI. VIRUS DISEASES OF PLANTS (ONLY THE MOST TYPICAL EXAMPLES ARE NOTED).

Diseases.	Transmission.	Incubation period.	Chief distribution.	Filtration of virus.	Inclusion bodies.	Lesions.	Susceptible plants.	Immunity.	Mortality.
Mosaic disease of tobacco plants.	Indirect; direct (experimental).	12–15 days	General	Filterable	Present	Leaves, flower, shoots, etc.	Nicotiana, petunia, daturia, capsicum, etc., tomato, potato.	Natural immunity exists in plant families.	Allard has never seen a plant recover.
Mosaic disease of tomato and potato plants.	do	2–3 weeks	do	do	do	Leaves, stems, tubers, etc.	Tomato, potato, tobacco.	do	Plants do not recover.
Mosaic disease of cucumbers.	do	4–5 days; rarely later than 8–9 days.	do	do	No report	Leaves, stems, fruits, etc.	Wild cucumber, petunia (?) Solanaceæ (?) Leguminoceæ (?)	do	Do.
Mosaic disease of lettuce, cabbage, mustard, turnip, spinach.	do	20–30 days	do	Probably filterable.	do	Leaves, flower, etc.	Plants mentioned in first column.	do	Do.
Mosaic disease of sugar cane.	do	3–6 weeks; variable.	United States; Porto Rico; Philippines; Cuba; Hawaii; etc.	do	Present	Blades, shoots, etc.	Many varieties of sugar cane.	Japanese and Argentine varieties immune.	Do.

TABLE 1.—*Classification of filterable virus and rickettsia diseases according to host susceptibility*—Continued.

VII. THE BACTERIOPHAGE.

Strain of bacteriophage.	Source.	Filtration.	Transmissible in series.	Destroyed by ultra-violet light.	Author.
Bacillus dysenteriæ Shiga	Stools of man and animals; river and water; soil.	Filterable	Transmissible	Killed (Zoeller)	D'Herelle.
Bacillus dysenteriæ Hiss	do	do	do	No report	Do.
Bacillus dysenteriæ Flexner	do	do	do	do	Do.
Bacillus gallinarum	Excreta of domestic animals	do	do	do	Do.
Pasteurella bovis	Excreta of buffaloes	do	do	do	Do.
Bacillus pestis	Excreta of rats; in buboes of man	do	do	do	Do.
Bacillus typhosus	Excreta, urine, and blood of patients; river water.	do	do	do	Do.
Bacillus paratyphosus A	do	do	do	do	Do.
Bacillus paratyphosus B	Excreta of man and animals	do	do	do	Do.
Bacillus suipestifer	Stools of normal man and horses	do	do	do	Do.
Bacillus enteritidis	Excreta of animals	do	do	do	Do.
Bacillus typhi-murium	Excreta of white and gray rats	do	do	do	Do.
Bacillus coli	Intestinal tract of all animals; river water.	do	do	Killed [1]	Do.
Bacillus Friedlander	Stool of infected patient	do	do	No report	Caublot.
Bacillus flacherie	Intestines of silkworms	do	do	do	D'Herelle.
Bacillus proteus	Stools from cases of infantile cholera	do	do	do	Do.
Bacillus swine fever	Old mixed culture	do	do	do	Bürgers and Bachmann.
Bacillus diphtheriæ	Excreta from immunized horses	do	do	do	D'Herelle.
Bacillus subtilis	Stool from dysentery patient	do	do	do	Do.
Vibrio cholera	Stool of patient; old cultures	do	do	do	Do.
Staphylococcus	Old cultures	do	do	do	Twort, Gratia.
Enterococcus	Fæces of horses	do	do	do	Beckerich and Hauduroy.
Streptococcus	Excreta of horse; old cultures	do	do	do	D'Herelle, McKinley, Piorkowski, Eichhorn.

Nodule bacteria of plants	Plant nodules	do	do	Killed in 2.5 hours	Gerretsen, Gryns, Sack, and Söhngen.
Bacillus pyocyaneous [i]	Old cultures	do	do	No report	Quiroga, Hadley.
Bacillus anthracis (pseudo) [j]	Old stock cultures	Not filterable	do	do	Monterio, Kraus, Gomez, Brown-Basaca.
Thermophilic bacillus T60	River water	Filterable	do	do	Koser.
Psychrophilic bacteria	Sewage	do	do	do	Elder and Tanner.
Bacillus pullorum	do	do	do	do	Hadley.
Staphylococcus fecalis and *S. lacticus*.	Pulp chamber of infected tooth	do	do	do	Hadley-Dabney.
Certain capsulated bacteria	Sewage	do	do	do	Hadley.

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[i] Fisher and McKinley, 62 minutes 1:10 dilution of bacteriophage active 1:10,000,000.

[j] According to d'Herelle this is not a true bacteriophage.

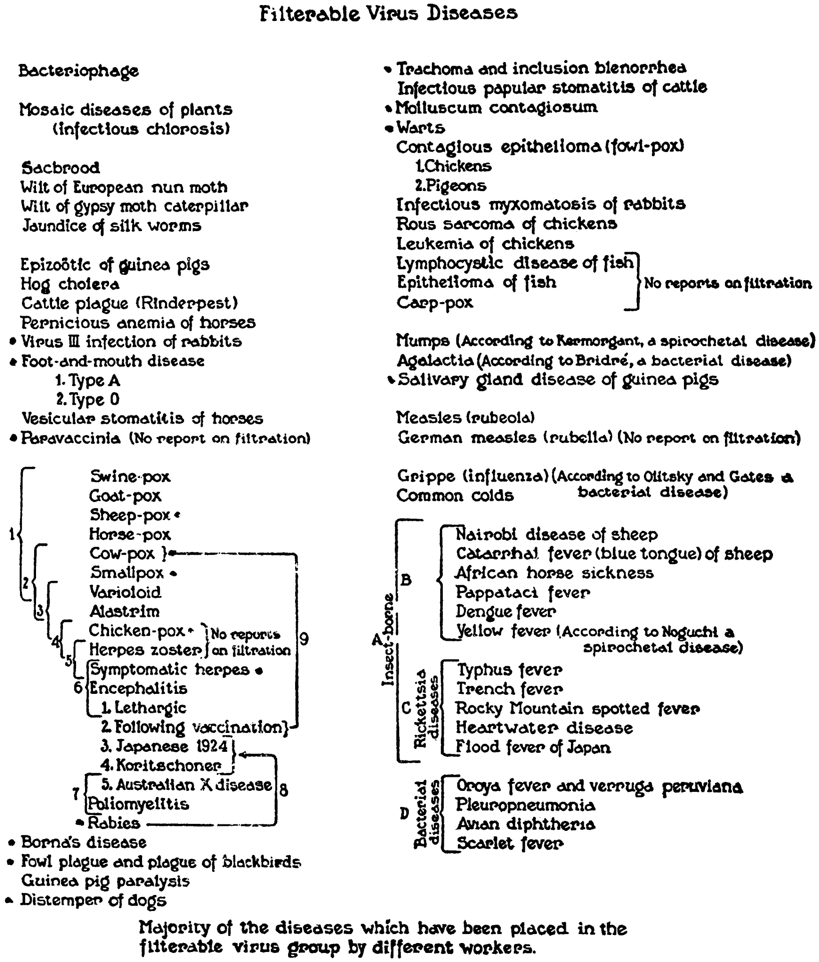
TABLE 2.—*Wolbach's chart of filterable virus diseases (1912).*

[This chart is a modification of Wolbach's chart as it appears in Hiss and Zinsser's Text Book of Bacteriology.]

Disease.	Transmission.		Occurrence.
	Direct.	Indirect.	
Molluscum contagiosum.	Direct contact		Man.
Dengue fever		*Culex fatigans*[a]	Do.
Verruca vulgaris, filtrability?	(?)		Do.
Trachoma, filterability?	Direct		Do.
Poliomyolitis	Unknown; probably nasal, etc., discharges.	Indirect by stable fly.	Do.
Measles filterability claimed by Goldberg and Anderson.	Direct		Do.
Scarlet fever, filterability claimed by Cantacuzene and Bernhardt but doubtful.	Probably direct		Man, chimpanzee.
Foot-and-mouth disease.	Direct		Man, cattle, swine.
Rabies	Direct by bite with saliva.		Man and all mammals; birds.
Variola and vaccinia	Direct		Man, cattle; transmitted to monkeys, rabbits.
Pleuropneumonia of cattle.	do		Bovine species.
African horse sickness		Probably insects; mosquitoes.	Horses.
Sheep pox	Direct		Sheep.
Cattle plague	Food contaminated with excreta.		Cattle.
Hog cholera	Direct		Hogs.
Swamp fever of horses		Probably indirect	Horses.
Agalactia of sheep and goats.	Contact		Sheep, goats.
"Blue tongue"	(?)	(?)	Sheep.
Guinea-pig epizoötic	(?)	(?)	Guinea pigs.
Guinea-pig paralysis	(?)	(?)	Do.
Novy's rat disease	(?)	(?)	Rats.
Fowl pest	Fæces		Pheasants, sparrows, geese.
Fowl diphtheria	Contant exudates, etc.		Fowl.
Rous's chicken sarcoma.	(?)	(?)	Chickens.
Pappataci (three-day fever).	Unknown	Phlebotomus papatassii.	Man.
Influenza, claimed by Yamanouchi, Olitsky and others.	Direct		Do.
Mumps, claimed by Wollstein.	do		

[a] It is known that dengue fever is transmitted by *Aëdes ægypti.*

TABLE 3.—*Rivers's chart (1927).*



Filterable Virus Diseases

Bacteriophage
Mosaic diseases of plants (infectious chlorosis)
Sacbrood
Wilt of European nun moth
Wilt of gypsy moth caterpillar
Jaundice of silk worms
Epizoötic of guinea pigs
Hog cholera
Cattle plague (Rinderpest)
Pernicious anemia of horses
• Virus III infection of rabbits
• Foot-and-mouth disease
1. Type A
2. Type 0
Vesicular stomatitis of horses
• Paravaccinia (No report on filtration)
Swine-pox
Goat-pox
Sheep-pox •
Horse-pox
Cow-pox •
Smallpox •
Varioloid
Alastrim
Chicken-pox •
Herpes zoster
No reports on filtration
Symptomatic herpes •
Encephalitis
1. Lethargic
2. Following vaccination
3. Japanese 1924
4. Koritschoner
5. Australian X disease
Poliomyelitis
• Rabies
1
2
3
4
5
6
7
8
9
• Borna's disease
• Fowl plague and plague of blackbirds
Guinea pig paralysis
• Distemper of dogs
• Trachoma and inclusion blenorrhea
Infectious papular stomatitis of cattle
• Molluscum contagiosum
• Warts
Contagious epithelioma (fowl-pox)
1. Chickens
2. Pigeons
Infectious myxomatosis of rabbits
Rous sarcoma of chickens
Leukemia of chickens
Lymphocystic disease of fish
Epithelioma of fish
Carp-pox
No reports on filtration
Mumps (According to Kermorgant, a spirochetal disease)
Agalactia (According to Bridré, a bacterial disease)
• Salivary gland disease of guinea pigs
Measles (rubeola)
German measles (rubella) (No report on filtration)
Grippe (influenza) (According to Olitsky and Gates a bacterial disease)
Common colds
A
Insect-borne
B
Nairobi disease of sheep
Catarrhal fever (blue tongue) of sheep
African horse sickness
Pappataci fever
Dengue fever
Yellow fever (According to Noguchi a spirochetal disease)
C
Rickettsia diseases
Typhus fever
Trench fever
Rocky Mountain spotted fever
Heartwater disease
Flood fever of Japan
D
Bacterial diseases
Oroya fever and verruga peruviana
Pleuropneumonia
Avian diphtheria
Scarlet fever

• Majority of the diseases which have been placed in the filterable virus group by different workers.

BIBLIOGRAPHY

1. PROWAZEK, Chlamydozoa. II. Gelbsucht der Seidenraupen. Arch. f. Protistenk. 10 (1907) 359.
2. LIPSCHÜTZ, Filterierbare Infektionserreger. Kolle and Wassermann's Handbuch der pathogenen Mikroörganismen 8 (1913).
3. SIMON, Physiological Reviews 3 (1923) 483–508.
4. MACCALLUM, Medicine 5 (1926) 59–75.
5. RIVERS, Journ. of Bact. 14 (1927) 217 (lit.).
6. POLLENDER (1849). Quoted from Zinsser's Text Book of Bacteriology 5th ed. (1922) 773.
7. LOEFFLER and PFEIFFER, Zeit. f. Hyg. 23 (1886).

8. Iwanowski (1892). Ueber zwei Krankheiten der Tabakspflanze (abst.). In Beiheft Bot. Centbl. **3** (1893) 266. The original in Land-u. Forstwiss. Russian; see also Bull. acad. imper. d. sci. de St. Petersburg **13**: 237.
9. Beijerinck, Centbl. f. Bakt., O. **5** 27.
10. Frosch and Loeffler, Centbl. f. Bakt., O. **5** (1898).
11. Negri, Zeitschrift für Hygiene und Infektionskrankheiten **43** (1903) 507.
12. Borrel, Anal. de l'Inst. Pasteur (1903).
13. Heymann, Deutsch. med. Wochenschr. **35** (1909) 1692.
14. Linder, Wien. klin. Wochenschr. (1909) 1555 and 1659; v. Graefe's Arch. f. Ophthal. **84** (1913).
15. Landsteiner and Popper, Zeit. f. Immunitätsforch. (1909) 377; Die Heine-Medinsche Krankheit, Berlin (1911).
16. Flexner and Lewis, Journ. Am. Med. Assoc. **53** (1909) 1639; Journ. Exp. Med. **12** (1909).
17. Rous, Journ. Exp. Med. **12** (1910) 696–706; **13** (1911) 397–411.
18. Rous, Journ. Exp. Med. **18** (1913) 416–427.
    Rous and Murphy, Journ. Exp. Med. **19** (1914) 52–69.
19. Gye, Lancet **2** (1925) 109–116; **2** (1926) 989.
    Barnard, Lancet **2** (1925) 117–123.
20. Flexner and Noguchi, Journ. Exp. Med. **18** (1913).
21. Noguchi and Cohen, Journ. Exp. Med. **18** (1913) 572.
22. Da Rocha Lima, Centbl. f. Allgem. Pathol. u. pathol. Anat. Verhandl. d. deutsch path. Gessellsch. zu Marburg. **24** (1913) 409.
23. Noguchi, Journ. Exp. Med. **45** (1927) 175.
24. Lipschütz (1913). See reference 2.
25. Twort, Lancet **2** (1915) 1241.
26. D'Herelle, The Bacteriophage (translation 1922); Immunity in Natural Infectious Diseases (1924); The Bacteriophage and Its Behavior (1926). Williams and Wilkins Co., Baltimore.
27. Noguchi, Journ. Exp. Med. **29** (1919) 547–596; **30**: 87.
28. Sellards, Am. Journ. Trop. Med. **7** (1927) 71.
29. Strauss and Lowe, Journ. Am. Assoc. **73** (1919) 1056.
30. Levaditi, Harvier and Nicolau, C. R. Soc. de Biol. **85** (1921) 161, 213, 287; **89** (1923) 984; **90** (1924) 1372; Ann. Inst. Pasteur **36** (1922) 63; C. R. Acad. d. Sc. **177** (1923) 985.
31. McKinley and Holden, Journ. Infect. Dis. **39** (1926) 441–450.
32. McKinley and Holden, Archives of Path. and Lab. Med. **4** (1927) 155–161.
33. Olitsky and Gates, Journ. Exp. Med. **33** No. 2 and 3 (1921) 125.
34. Noguchi, Journ. Am. Med. Assoc. **89** (1927) 740–742.
35. Wolbach, Quoted from Park and Williams, Pathogenic Microörganisms, 7th ed. (1920) 530.
36. Wherry, loc. cit.
37. V. Esmarch, loc. cit.
38. Borrel, loc. cit.
39. Calmette and Valtis, Ann. de med. **19** (1926) 553.
40. Mellon and Jost, Soc. Exp. Biol. and Med. **24** (1927) 743.
41. Fabry, Bruxelles Medical **7** (1927) 596.

42. De Potter, C. R. de la Soc. de Biol. **96** (1927) 138.
43. Burnet, Arch. de l'Inst. Pasteur de Tunis **15** (1926) 292.
44. Lewis, Journ. Exp. Med. **45** (1927) 277.
45. Noguchi, Journ. Exp. Med. **44** (1926) 1–10.
46. Kramer, Journ. Infect. Dis. **40** (1927) 343.
47. Zinsser and Fei-Fang Tang, Journ. Exp. Med. **46** (1927) 357.
48. Levaditi and Nicolau, C. R. Acad. **176** (1923) 717.
49. Levaditi, Nicolau, and Galloway, C. R. Acad. **182** (1926) 247.
50. Olitsky and Boëz, Journ. Exp. Med. **45** (1927) 673–685.
51. Pierce and McKinley, unpublished data.
52. Rawlins, Science **65** (1927) 398.
53. Mines, Koll. Chem. Beihefte **3** (1912) 191–236.
54. Sherman, Caldwell, and Adams, Journ. Am. Chem. Soc. **48** (1926) 2947–2956.
55. Johnson-Blohm, Zs. Physiol. Chem. **82** (1912) 178–208.
56. Bronfenbrenner and Muckenfuss, Proc. Soc. Exp. Biol. and Med. **24** (1926) 372.
57. McKinley and Holden, Proc. Soc. Exp. Biol. and Med. **24** (1927) 595–598.
58. Le Fèvre, C. R. de la Soc. de Biol. **96** (1927) 206–207.
59. Grasset, C. R. de la Soc. de Biol. **96** (1927) 839.
60. Novy, Rhoem, and Soule, Journ. Infect. Dis. **36** (1925) Nos. 2, 3, 4.
61. Bronfenbrenner, Science **63** (1926) 51.
62. McKinley and Coulter, Proc. Soc. Exp. Biol. and Med. **24** (1927) 685–688.
63. McKinley and Holden, Journ. Infect. Dis. **39** (1926) 451–456.
64. McKinley, Fisher, and Holden, Proc. Soc. Exp. Biol. and Med. **23** (1926) 408–412.
    Fisher and McKinley, Journ. Infect. Dis. **40** (1927) 399–403.
65. Bronfenbrenner and Korb, Journ. Exp. Med. **42** (1925) 483.
66. Von Glahn and Pappenheimer, Am. Journ. Path. **1** (1925) 445.
67. Cowdry, Journ. Bact. **13** (1927) 20–21.
68. Wolbach, Journ. Med. Res. **27** (1912) 1.

CHAPTER II

FILTERABLE VIRUS DISEASES OF MAN AND ANIMALS

VARIOLA: SMALLPOX

POCKENKRANKHEIT (GERMAN); VARIOLE (FRENCH); VAJUOLO (ITALIAN)

Definition.—Vaughan(1) defines smallpox as "an acute, specific, infectious disease of unknown origin, characterized by sudden onset; usually with severe chill, accompanied by rapidly rising temperature and followed by a more or less general eruption which passes through papular, vesicular, and pustular stages, frequently leaving more or less permanent scars." To this concise and clear-cut definition may be added that there are four clinical types; namely, discrete, confluent, hæmorrhagic, and modified; and that the greater the eruption the more severe is the disease.

History.—There seems to be little doubt that this disease originated in the Orient. In ancient times the people of India knew the value of performing inoculation for the disease. Vaughan states that in one of the ancient Indian medical books there are special prayers which were repeated by the Brahman priests while performing the operation of inoculation for this disease. According to this author the practice of inoculation was introduced into China from India during the third century before Christ. Arabian physicians described the disease in the ninth and tenth centuries, and it is said to have existed in Egypt about 1200 B. C. The spread of smallpox over the world was extremely slow. Presumably it spread from India to China, then through western Asia into Turkey and Europe since it was known in England in the sixteenth century; and from Europe it spread to America.

Distribution.—As in olden times, smallpox is still widely distributed over the face of the entire earth wherever the human species exists. It has been estimated that sixty million people died of this disease in the eighteenth century.(2) It is still a serious affliction of the human race everywhere and at times assumes epidemic proportions causing untold suffering and loss of life.

Incubation period.—The period of incubation in smallpox varies from nine to fifteen days and on the average is twelve days. Fourteen days is usually given as the time which elapses between exposure and the appearance of the eruption although the prodromal symptoms appear earlier.

Symptoms.—The prodromal symptoms, which are the first indication of the disease, are characterized by a rise in temperature which may be followed by a severe or light chill, nausea and vomiting, headache and backache, and a slight measleslike or scarlatiniform rash may appear. Usually the characteristic rash develops on the third day following the chill and rise in temperature. The papules as they begin to appear present a "shotty" feel and usually are discrete but may be confluent. Three days later the stage of vesiculation appears and the lesions contain clear serum. The smallpox vesicle exhibits a characteristic central depression or umbilication and gradually becomes turbid and passes into the suppurative or pustular stage. This usually occurs on the sixth day. The lesions may remain discrete or become confluent. Schamberg(3) has described one patient upon whom 26,791 pustules were counted and estimated the total amount of pus carried by the patient to be about 5 quarts. On another case he estimated that there were 40,000 pustules.

During recovery the pustules dry and the lesions pass through the stages of desiccation, crusting, and decrustation. Scars ultimately take their place and remain as a reminder of this dread disease.

Animals susceptible to the pox virus.—The question of the interrelationship between all the forms of pox as they occur in man, animals, fowls, and fishes is a mooted one. Some authors take the view that pox per se is a disease entity and that its appearance in different species is modified by two factors; namely, the response to pox virus varies with the species and the virus itself is modified by the species. Certainly the etiological relation of human and animal pox is not sufficiently established to warrant general statements in the matter. From an anatomical point of view the etiological identity of the different forms of pox is indicated. The disease appears in man and in sheep as a general infection, but the lesions are distinctly localized to certain parts of the body in cattle and goats. Furthermore, the disease in man and sheep frequently becomes epidemic in form, while the disease is limited in other animals. That there is a very close relationship between human pox and cowpox is beyond question. However, only local lesions are produced in man when inoculated with cowpox, and only in a few experimental cases have lesions developed in cattle as a result of inoculation with human pox. The close relation between these two forms of pox is proven, however, by the fact that cowpox virus produces a high immunity against smallpox in man. The general view may be taken that all forms of pox are identical; and such a concept enables one better to understand the disease in its broadest sense, but experimental data are lacking for this viewpoint at the present time. It is difficult, for example, to explain why pox in sheep spreads naturally to other sheep but does not pass to the closely related goats; nor is it dangerous for man. If such a general view is to be considered we must assume that the virus of pox is modified in the different species and in that way account for its paradoxical manifestations. If such a view is acceptable then one may state that pox virus is pathogenic for many different species; such as, man, sheep, cows, goats, horses, swine, fowls, and certain fishes (carp). We must further assume that, due to a variety of unknown conditions, aberrant forms of the disease do occur, and that these atypical conditions fall in the same category as true pox infections, but in a modified form.

For the present we are unable to accept this view notwithstanding its convenience, for there are too many contradictory facts existing. Suffice it to say that a form of pox is present in many different forms of animal life and within certain limits only do we understand the interrelation between them. Beyond this no general statements are permissible in the light of present knowledge, and broader interpretations must await future investigation.

The pox virus.—It is definitely known that the vesicular contents of pox lesions contain a virus that is filterable under high pressure through the less-dense porcelain filters, and for this reason the pox virus is classified with the group of filterable microörganisms.

In the epithelial cells of affected parts of the skin, and between the cells, there have been demonstrated by Prowazek,(4) Borrel,(5) Casagrandi,(6) Paschen,(7) Volpino,(8) and others, extremely small bodies that are from 0.2 to 0.5 micron in size, Gram negative, and stain with Loffler's flagella stain and with Giemsa's. Borrel described bodies in sheep pox in 1903 which have since been designated sheep-pox bodies. Prowazek has designated the small bodies found in pox lesions in man and in vaccinia as Chlamydozoa, but they are also referred to as Prowazek's elementary bodies. Later these bodies become larger and are thought to represent a later stage in the development of the virus. These larger forms are frequently spoken of as Prowazek's initial bodies. Lipschütz has designated them as *Strongyloplasma variola-vaccinæ.* The initial bodies are also known as Guarnieri bodies, named after an investigator who thoroughly studied them,(9) although they were first studied by Weigert in 1874 and later by Pfeiffer in 1886. The Guarnieri bodies (*Cytorrhyctes vaccinæ,* vaccine bodies) are spherical or half-moon-shaped bodies consisting of chromatin or plastin substance. They are usually found lying close to the nucleus of the cell and rarely in the cytoplasm. In fresh preparations there appears to be an amœboid movement. The exact nature of these bodies is unknown though it has become somewhat a general belief that they represent the reaction products of the cell plasma to the penetrating virus. According to this concept it is reasoned that the minute infectious agent, Prowazek's elementary body, enters the cell substance and there becomes inclosed by a plastin substance which is produced by the plasma of the cell as a protective reaction against the assault of the invader. Further the vaccine body again breaks down, the initial body passes into the plasma of the cell, there disseminates and separates into elementary bodies. These elementary bodies may then, as Prowazek has argued, reach the outside world after the epithelial cell is destroyed and be capable of again infecting a susceptible host.

According to some authors vaccine bodies represent only migrated or changed leucocytes; still others believe that they are products of the medullary layer of the plasma of the cell or that they are fragments of the nucleus; while others attribute their origin to the centrosome or consider them as products of endogenic cell rejuvenation, cellular inclusions of the second order.

Whatever their origin or their nature they are characteristic of the disease. The pox virus may be propagated through inoculation of the cornea of rabbits, and Guarnieri bodies may be demonstrated in the lesions. According to Schultz(10) these bodies in inoculated rabbit's cornea are derived from the cytoplasm and are formed in response to infection or poisoning of the cells. This author concludes that nothing suggests the presence of the virus of smallpox in the Guarnieri body. Gracium and Oppenheimer(11) have described the cultivation of vaccinia "granules" in vitro with embryonic tissues and state that they remain alive for as long as seventy-one days as tested by rabbit corneal inoculation. In 1916 Paul (19) demonstrated that following the scarification of the rabbit's cornea with the pustular contents of cowpox and the immersion of the enucleated eye after forty-eight hours for a few minutes in sublimate alcohol, the lesions stand out prominently as white elevations on a dull milky-white background. This procedure has since been known as the Paul test. Toomey and Gammel (20) recently have reported a series of eighty cases of smallpox in which they were able to get 45 per cent positives and conclude that the test, when present, is pathognomonic of the disease.

Bacteria are frequently associated with pox virus. Streptococci, staphylococci (*aureus* and *citreus*), and other bacterial forms have been reported.

Immunity.—It is well known, and was recognized by ancient peoples, that one attack of smallpox confers an immunity to the disease. While artificial immunity may be produced by vaccination, the immunity so produced does not last indefinitely.

Vaccination against smallpox.—In ancient times in India the practice of inoculation in smallpox was the custom. During those times the danger of transmitting syphilis and other diseases by such an operation was only partially recognized, though the records are doubtful on this point. As smallpox spread over the world from India by way of Asia, so did the custom of performing inoculation against the disease. This is of particular interest since this method of immunization preceded the present method of vaccination and had a direct bearing upon its development. According to Vaughan, England was not informed of the practice of performing inoculation until 1713. Lady Montagu having learned the value of inoculation in the prevention of smallpox in Constantinople had her own son immunized in this manner, and returning to England in 1718 she supported the work of several English physicians who were interested in the prevention of smallpox. Several convincing experiments were performed upon convicts, and the Princess of Wales had six charity children inoculated. The method became popular for a time but gradually became unpopular again following several severe accidents that resulted from faulty methods employed. From this time until 1840 inoculation was practiced with more or less success in England and America, though Jenner's classical experiments on vaccination with cowpox virus were performed in 1796.

Jenner himself had been inoculated against smallpox when he was a boy and had noticed that persons who had previously had cowpox were not susceptible to inoculation. His experiments as reported included ten cases all of whom had had cowpox. The length of time that had elapsed between having cowpox and being inoculated with smallpox varied from nine months to thirty-eight years. None of the inoculations took. This paper was published in 1798. It was clearly recognized by Jenner that neither natural smallpox nor vaccination with cowpox virus was an absolute and permanent protection against the disease, but while vaccination did not give permanent immunity against smallpox he knew that vaccination might modify the course and severity of second attacks.

In America the results of Jenner's experiments were quickly applied. A group of nineteen boys were vaccinated with cowpox in Boston in 1802, and about three months later twelve of the vaccinated boys were inoculated with smallpox. None of them developed smallpox. Two unvaccinated controls developed the disease after being inoculated with the same virus. From this time vaccination became the scientific and popular method for preventing smallpox.

Rosenau(2) defines vaccination as the introduction of vaccine virus into the skin with the object of inducting cowpox (vaccinia) in order to prevent smallpox (variola). A true "take" is very characteristic and is known as a primary take. Modified reactions may appear in persons previously vaccinated or in those who have had the disease.

Following about three days incubation a papule appears which develops in another three-day period into a vesicle. In the next three-day period the vesicle passes into the pustular stage, and within three days the pustule is fully developed. Desiccation and decrustation follow. While this is the typical course in vaccination there are deviations now and then. Associated with the skin manifestations there are frequently mild or, in some cases, very severe constitutional symptoms. Rise in temperature, headache, backache, loss of appetite, nausea, and vomiting may accompany the process.

Methods of vaccination.—Many methods have been employed in vaccinating against smallpox. Chief among these methods are the puncture method, intradermal vaccination, scarification, and the incision method. Recently the pressure method has been advocated by Thomas and Bull(12) and seems to offer several advantages over the usual methods employed.

Preparation of cowpox vaccine.—In the United States in 1902 a law was passed regulating the manufacture of vaccine virus. This law requires the licensing of manufacturers of vaccine virus, and through periodic government inspection a uniform product is assured to the public. The law also requires manufacturers to examine each lot of vaccine by bacteriologic methods to determine the number and the kind of bacteria present. Special tests which include animal inoculation and cultural methods are specifically required to determine if pathogenic organisms such as streptococci, tetanus organisms, etc., are present.

The virus of vaccinia is more concentrated in the epithelial cells though it is also present in the vesicular contents. Both the lymph and the pulp are employed in preparing vaccine. Bovine virus has been used since the time of Jenner because it is always obtainable and was later found by Monckton Copeman (13) in 1891 to be easily purified when preserved in glycerin. While the vaccine virus is very resistant to the action of glycerin, the nonspore-bearing bacteria are for the most part destroyed.

Young calves are usually used for propagation of the virus, and in the East the carabao is employed. The animals to be used are first quarantined for a week or ten days to determine the absence or presence of other infections. When it is known that the animals are free from infection, they are thoroughly cleaned, and the abdomen is shaved and sterilized with a mild germicide. The seed virus is then rubbed into the superficial incisions that are made in the skin. The animals are then kept in screened isolation rooms, and about the fifth to the seventh day the vesicles are removed. The animal is killed and autopsied to rule out any infectious lesions other than vaccinia. The virus may be dried and used when needed, or it may be glycerinated—the latter is to be preferred since the former cannot be purified. The dried virus may remain viable for a long period, but in time the glycerinated virus loses its potency, and for that reason old preparations should not be employed in vaccination. Though many modifications are practiced in various laboratories in the preparation of vaccine virus, the fundamental considerations are the same in all methods as here described. Full and detailed descriptions of the different methods employed are to be found in the standard texts.

It is possible that future investigations upon the cultivation of vaccine virus may result in the use of pure virus for the purpose of vaccination. MacCallum and Oppenheimer(14) have already separated the "granules" from the rest of the vaccine by differential centrifugalization of the virus in a solution of glycerin and Locke's solution. Parker(15) has presented evidence of its cultivation in tissue culture, and the work of Cracium and Oppenheimer on cultivation has already been referred to. Aside from the purely practical aspects of this work such research is very important to the whole question of the nature of the filterable viruses and represents a marked advance in their study.

While it is well recognized that smallpox is primarily a disease that localizes in the skin and manifests itself by producing lesions there, it is thought that normal infection also takes place through the respiratory tract. That the skin as an organ can be infected directly is amply proved by inoculation and vaccination. Though it is not common, a generalized vaccinia sometimes results from vaccination. This has been commented on by students of local immunity, and to their minds the process of vaccination consists in immunizing the skin which is the susceptible organ of the body to this virus. Ledingham(16) has recently shown that the infiltration of an area of the skin with ink and subsequent vaccination suspends all response to the naked eye. He suggests that the local defense is increased by stimulating the reticulo-endothelial cells to proliferate. Schöbl(17) in his most recent comprehensive study on yaws in the Philippines suggests an analogy in the mechanism of immunity in yaws and that in smallpox. The tendency of modern research on smallpox is very promising of fruitful results in as much as the effort is being directed away from empiricism which has for so long dominated the study of this disease. New facts concerning this disease may throw much light upon the nature of other filterable virus diseases.

VARIOLOID

Varioloid is usually defined as a modified and mild form of smallpox occurring in a patient who has had a previous attack of smallpox or has been vaccinated.

Vaughan(1) draws attention to this condition, and in his opinion the name is very unfortunate since it suggests a disease resembling smallpox but different from it. He further states that while varioloid is not of much danger to the patient, since it usually runs a mild course, nevertheless it is smallpox and as such is extremely dangerous to persons with whom the patient comes in contact. No longer do we accept the dictum that one attack of smallpox or vaccination protects the patient for life, since it is well known that one may have smallpox more than once and that vaccination protects the individual against smallpox for only a variable period of time. The characteristic symptoms and lesions are all present in varioloid, though as a rule there are fewer lesions and they do not penetrate as deeply as the lesions of true smallpox.

PARAVACCINIA

Paravaccinia is an anomalous response to vaccination with calf lymph which has been described as occurring in Vienna and other parts of Austria. It has been thought that some of the calf lymph produced at one of the vaccine laboratories in Austria has become contaminated with an aberrant form of vaccine virus.

As has already been mentioned the Guarnieri bodies in pox lesions have been described as occurring in the cytoplasm of the epithelial cells. In paravaccinia Lipschütz(18) has described inclusion bodies as occurring not only in the cytoplasm of the cell but also in the nucleus. While Guarnieri bodies have been described as occurring in the nucleus, they are very rarely found in this location.

ALASTRIM: AMAAS: KAFFIR MILK POX

Alastrim is regarded as an aberrant form of smallpox prevalent in certain parts of Africa, the West Indies, and South America, and is characterized by its short period of incubation and its low mortality.

Aberrant forms of smallpox are found in almost every epidemic and from the standpoint of the public health must be regarded with the same seriousness as true forms of the disease. Alastrim is no exception to this rule. This disease is prevented by vaccination as is variola vera. It may be noted that inclusion bodies have been described for this form of smallpox by Castellani and Chambers.

BIBLIOGRAPHY

1. Vaughan, Epidemiology and Public Health. C. V. Mosby Co., St. Louis 1 (1922).
2. Rosenau, Preventive Medicine and Hygiene, 5th ed. D. Appleton & Co., New York and London (1927) 27.
3. Schamberg, Diseases of the Skin and the Eruptive Fevers. W. B. Saunders Co., Philadelphia (1917).
4. Prowazek, Handbuch der pathogenen Protozoen, Leipsic 1 (1912) 122; Arch. f. Prostistenkunde 10 (1907).
5. Borrel, Annal. de l'Inst. Pasteur (1903).

6. CASAGRANDI. Livre Jubilaire de A. Celli, Rome (1912) 709.
7. PASCHEN, Münch. med. Woch. **55** (1908) 2494; **56** (1909) 2004.
8. VOLPINO, Centralbl. f. Bakt. **49** (1909) 197.
9. GUARNIERI, Arch. per le Scienze med. **16** (1892) 403 (fig. 1).
10. SCHULTZ, Ztschr. f. Hyg. u. Infectionskrankh. **105** (1925) 1.
11. CRACIUM and OPPENHEIMER, Journ. Exp. Med. **43** (1926) 815.
12. THOMAS and BULL, Journ. Am. Med. Assoc. **38** (1927) 1879.
13. COPEMAN, Tr. Internat. Cong. Hyg. (1891).
14. MACCALLUM and OPPENHEIMER, Journ. Am. Med. Assoc. **78** (1922) 6.
15. PARKER and NYE, Am. Journ. Path. **1** (1925) 325.
16. LEDINGHAM, Brit. Journ. Exp. Path. **8** (1927) 12; **5** (1924) 332.
17. SCHÖBL, Philip. Journ. Sci. **35** (1928).
18. LIPSCHÜTZ, Arch. f. Derm. u. Syph. **127** (1922) 193.
19. PAUL, Wien. klin. Wchnschr. **19** (1916) 996.
20. TOOMEY and GAMMEL, Journ. Infec. Dis. **41** (1927) 28–31.

OTHER REFERENCES

ALDERSHOFF and BROERS, Ann. de l'Inst. Pasteur **20** (1906) 779.

ARNDT, Centralbl. f. Bakt. **47** (1908) 237.

BOING, Arb. a. d. Reichsgesundheitsamte **52** (1920) 615.

BLANC and CAMINOPETROS, C. R. Soc. de Biol. **88** (1923) 1020; **89**: 38.

CAMUS, Bull. de l'Acad. de Med. **40** (1923) 79.

CALKINS, Journ. Med. Res. **11** (1904) 136.

COPEMAN and MANN, Twenty-eighth Ann. Rep. Local Govt. Board, London (1899) 505.

COUNCILMAN, MAGRATH, and BRINCKERHOFF, Journ. Med. Res. **11** (1904) 12.

COWDRY, Journ. Exp. Med. **36** (1922) 667.

EWING, Journ. Med. Res. **12** (1904) 508; **13** 233.

FRIEDEMANN and GINS, Deutsch. med. Woch. **43** (1917) 1159.

GINS, Zeit. f. Hyg. u. Infekt. **95** (1922) 255.

GOERTTLER, Zeit. f. Immunitatsforsch. **38** (1923–24) 357.

GORDON, Med. Research Council, Special Report No. **98** (1925).

HACH, Zeit. f. Hyg. u. Infekt. **104** (1925) 569.

KRUMBACH, Zeit. f. Immunitatsforch. **38** (1923–24) 1.

LEVADITI and NICOLAU. C. R. de Soc. de Biol. **35** (1921) 345; **88** (1923) 571; **89**: 484; Ann. l'Inst. Pasteur **37** (1923) 1.

LUCKSCH, Centralbl. f. Bakt. u. Par., i, Orig. **96** (1925) 309.

NAKAGAWA, Zeit. f. Immunitatsforsch. **42** (1925) 409.

NODAKE, Zeit. f. Immunitatsforsch. **41** (1924) 52.

PARKER, Journ. Med. Res. **44** (1923–24) 645.

POPPI and HERZBERG, Centralbl. f. Bakt. u. Par., i, Orig. **95** (1925) 211.

SCHNEIDER, Zeit. f. Immunitatsforsch. **38** (1923–24) 271.

SCHULTZ, Zeit. f. Hyg. u. Infekt. **105** (1925) 1.

RENAUT, Ann. de Derm. et de Syph. **2** (1881) 1.

UNGERMANN and ZUELZER, Arb. a. d. Reichsgesundheitsamte **52** (1920) 41.

PROWAZEK and BEAUREPAIRE-ARAGOA, Münch. med. Woch. **55** (1908) 2265.

WASIELEWSKI, Centrabl. f. Bakt. **21** (1897) 901.

WOODCOCK, Journ. Roy. Army Med. Corps **37** (1921) 418.

VARIOLA VACCINIA: COWPOX

KUHPOCKEN (GERMAN); VACCINIA (FRENCH); VAJULA VACCINO (ITALIAN)

Definition.—Cowpox, or vaccinia, is an acute, febrile, infectious disease of cattle, characterized by a vesicular exanthema. This disease is closely related to smallpox in man since vaccination with it will protect man from smallpox.

History.—The history of smallpox has already been considered. In as much as cowpox, in most cases, can be traced to an infection from human beings affected with pox, the history of this disease and its virus can be traced back to the earliest records in which smallpox in man is described. We may consider cowpox then as a disease that has been known to the world since ancient times.

Distribution.—The distribution of cowpox runs parallel with that of smallpox and undoubtedly, according to the most authentic records, spread over the entire world from the original locus of smallpox in India.

Incubation period.—The usual incubation period in cowpox ranges from four to seven days but in some cases may be somewhat less than four days and slightly more than seven days.

Symptoms.—After an incubation period of four to seven days the disease begins with prodromal symptoms which may be so mild that they remain unnoticed. These symptoms consist in a slight rise of 0.5 to 1° in temperature, weakness, diminished appetite, and irregular rumination. The udder becomes sensitive, and the milk is thinner, of a low specific gravity, and coagulates more quickly. On the second to the third day pea-sized hard nodules appear upon the udder and teats. Within one or two days these nodules develop into reddish or bluish, or yellowish-white vesicles, frequently with a metallic luster. From the eighth to the eleventh day the vesicles begin to become purulent and a well-defined depression in the center is noticed ("Delle" navel). The pustules gradually dry up, and scabs are formed which later drop off leaving pitted depressions in the scarred tissue. There are rarely many vesicles, exceptionally more than twenty and frequently only a very few which may not develop simultaneously but appear at intervals representing different stages of the disease. Owing to mechanical conditions in milking the vesicles may be broken and as a result ulceration may take place which requires a month or more to heal. In such cases the milk becomes contaminated. In the male the lesions are to be found upon the scrotum. Generalized eruption may occur, but it is exceedingly rare. Strebel(1) has reported one case of generalized eruption in which lesions appeared on the udder, croup, rump, chest, and neck, and on the inner surface of the thigh.

In man a generalized eruption following vaccination with cowpox virus is occasionally seen, the eruption being particularly disseminated over the back. Such a generalized eruption is considered not uncommon in the Philippines.

As in smallpox, aberrant forms of cowpox frequently appear and complicate the diagnosis of this disease. At times these atypical forms resemble somewhat the exanthema of foot-and-mouth disease, but in this condition the vesicles are larger and do not develop from nodules and are present in the mouth. According to Hutyra and Marek(2) the benign, coital vesicular exanthema is readily recognized by the simultaneous affection of the genital organs.

If the vesicular contents are injected into the cornea of the rabbit, transparent proliferations may develop about the second day and Guarnieri bodies may be found in the fresh or stained preparations.

So-called "wind and stone pox" has been described by German authors, and this form of pox may be an aberrant form of true cowpox. The disease described by Ehrhardt(3) as false cowpox, pointed pox, may also fall in this classification. The "varicellen," water pox or wind pox, in which vesicles develop within only a few hours in children is probably not related to true pox in as much as no immunity against true pox is produced. Rudolph,(4) in writing of the benign "white pox," which occurs in tropical countries, states that the central depression in the pustule of this disease is absent and that it produces an immunity against vaccine virus which lasts for about six months. Perhaps this condition may also fall into the category with other aberrant forms of true cowpox and the virus in this case may be so modified that its antibody stimulating or antigenic qualities may be considerably reduced. That it produces immunity to cowpox virus even for so short a period is sufficient evidence that it is closely related biologically.

Animals susceptible to the virus of cowpox.—Vaccinia virus is capable of producing lessions in cattle (chiefly cows), in man, in the skin and cornea of rabbits, and in monkeys. According to Voigt(5) it is not possible to immunize cattle with sheep-pox virus against cowpox and vice versa. Likewise cattle are not susceptible to goat pox; but horses, buffaloes, camels, hogs, sheep, dogs, and fowls are all mildly susceptible to cowpox virus. Guinea pigs have been used for experimental purposes in the study of vaccine virus and are susceptible.

The virus of cowpox.—Negri(6) demonstrated in 1905 that vaccine virus is filterable through coarse porcelain filters. Considerable pressure is needed with these filters to demonstrate the filterability of this virus. It is not filterable through fine filters even when considerable pressure is employed. Negri filtered calf lymph through Berkefeld filters under three atmospheres and was able to produce with the filtrate a true vaccinia keratitis on the cornea of rabbits and lesions upon the udders of cows. It is known that a filtrate prepared from lymph that has stood for some time is much more infective than a filterate from fresh lymph, and according to Carini(7) this is explained upon the basis that the virus is contained for the most part within the cell, and after the lymph has stood awhile the cells disintegrate and liberate the virus. Nicolle and Adil-Bey(8) have shown that the infectivity of lymph may be increased by digestion with pancreatin. The morphology of the Guarnieri bodies has already been considered in the discussion of smallpox in man and cultivation experiments with vaccine virus by Parker, Parker and Nye, and Cracium and Oppenheimer were described. These authors apparently succeeded in cultivating this virus only when associated with living cells. In 1906 Proscher(9) reported the cultivation of pox virus upon artificial solid media. According to this author's report smeary deposits develop on solid media that do not contain microscopically visible microörganisms but which produce pox pustules in calves up to the third and the fourth passage. It is doubtful if these experiments actually represent cultivation. Up to the present time the best method of propagating the vaccine virus consists in infecting a susceptible animal.

Vaccine virus kept at a low temperature and protected from light and air remains virulent for months; room temperature rapidly attenuates it. It is destroyed in five minutes at a temperature of 58° C. The virus is very resistant to the action of glycerin but in time is attenuated by this reagent. Various disinfectants will, of course, destroy it.

Immunity in vaccinia.—While vaccination of lower animals against pox is possible it is rarely employed according to Kelser. The chief use of vaccine virus is to protect man from smallpox. This subject has been fully discussed under smallpox. Fréger(10) vaccinated a herd of cows with calf lymph used for man and produced typical pox pustules. These animals did not develop cowpox though they were part of an infected herd.

Control measures to prevent cowpox.—Control measures consist in isolation of infected animals, careful milking to prevent complications and greater contamination of the milk, and vaccination of healthy animals although the last is little practiced.

BIBLIOGRAPHY

1. Strebl, Schw. A. 40 (1898) 113.
2. Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Am. ed. by Mohler and Eichhorn. Alexander Eger, Chicago 1 (1926).
3. Ehrhardt, Schw. A., 81 (1896).
4. Rudolph, Münch. Med. Woch. (1911) 295.
5. Voigt, Zeit. f. Infkrkh. 6 (1909) 101.
6. Negri, Esper. sulla filtr. del virus vacc. Pavia (1905).
7. Carini, Centralbl. f. Bakt. 42 (1906) 325.
8. Nicolle and Adil-Bey, C. R. de Soc. Biol. 142 (1906) 1196.
9. Pröscher, Centralbl. f. Bakt. 40 (1906) 337.
10. Fréger, J. vét. (1906) 385.

For other references see Smallpox.

VARIOLA OVINA: SHEEP POX

SCABROT, SCHAFPOCKEN (GERMAN); CLAVELÉE, PICOTTE (FRENCH)
VAJUOLA PECORINO (ITALIAN)

Definition.—Sheep pox is an acute, febrile, infectious disease of sheep characterized by a papulo-vesiculo-pustular eruption with catarrhal symptoms involving the mucous membranes of the eyes and the respiratory passages.

History.—As smallpox in man, sheep pox was probably introduced from Asia and was probably known long before the first studies on the disease were made by Joubert and Rabelais toward the end of the sixteenth century. The infectiousness of sheep pox is said to have been established in 1763 by Bourgelat.(1) In 1868 Chauveau(2) published important investigations upon the etiology and pathogenesis of this disease, while Borrel(3) in 1903 first described the so-called "sheep pox bodies."

Distribution of sheep pox.—The chief distribution of sheep pox is in France and in the southern and eastern European countries. The disease is widely spread in Asia and in Africa but is noted in that part of the world for its benign course and is often unnoticed. However, it is reported that in 1909, in German Southwest Africa, where the disease was imported with Karakul sheep, in one district twenty-five thousand sheep died. In 1823 the loss of sheep in Austria alone was estimated at four hundred thousand animals. In 1910 in that country only a few sporadic cases were reported. Germany has been said to be free of the disease since 1909. There is no record of the disease ever having occurred in America or in Australia.

Incubation period in sheep pox.—The incubation period in sheep pox is usually from six to eight days and in some cases may be somewhat longer. In winter the time of incubation is usually slightly longer than in warm weather.

Symptoms.—The prodromal signs of the disease consist of a rise in temperature to from 41 to 42° C. which may be accompanied by chills. Along with the febrile conditions there is an acceleration of the pulse and respiration, loss of appetite, and catarrhal symptoms referred to the mucous membranes of the eyes and the respiratory tract. The eyes are half closed and a mucous discharge exudes. From the nose there is also a mucopurulent discharge. The tongue is coated, and the mucous membranes of the mouth and throat are inflamed.

After one or two days have elapsed there appear upon the skin near the eyes, on the cheeks, nose, and lips, on the vulva or prepuce, udder or scrotum, and on the tail and thighs round red areas which may be surrounded by a serous infiltration. Within twenty-four hours nodules appear in the center of these spots, and soon vesicles appear which contain a yellowish serous fluid. A depression develops in the center of each vesicle and there is a distinct zone of hyperæmia surrounding them. This development usually requires five or six days. On the sixth or seventh day these vesicles gradually change into pustules and the hyperæmia surrounding the lesion becomes more intense. Desiccation begins and crusts are formed that later drop off leaving a red scar that later turns white and is somewhat pitted.

With the eruption there is usually continued fever, which rises to its height during the development of the pustules. There is a constant profuse discharge from the eyes and nose, and foamy saliva hangs in strings from the corners of the mouth. During healing there is falling out of the wool and severe itching.

Lesions similar to those described for the skin usually develop upon the mucous membranes of the mouth and may even involve the pharynx.

Atypical forms of sheep pox occur in which there are no skin lesions at all and the animal exhibits only the catarrhal condition described above. In other forms, such as the so-called "stone pox" which occurs in Algeria, the skin lesions do not progress beyond the papule stage. Such a form of the disease appeared in Germany in 1905 and was described by Joest(4) and others. Such nodules often became ulcerated and even gangrenous. In some cases there developed keratitis followed by panophthalmitis, and blindness resulted. The constitutional symptoms remained severe as in the typical disease. It is thought that these abortive forms result from a high immunity that the animal has developed for the disease. The mortality in the German epidemic in 1905 is estimated to have been from 25 to 50 per cent of all animals affected. In some cases extensive hæmorrhages appear during the eruption, and as in smallpox this sign is regarded with great seriousness.

Complications frequently result in which the animal develops a catarrhal pneumonia, or the infection may involve the bones, particularly the nasal septum, and necrosis sets in. Other complications, such as suppurative lymphadenitis, metastatic abscess in internal organs, pleurisy, and necrosis of the tendons, have been noted.

Animals susceptible to the virus of sheep pox.—Sheep pox usually spreads only among sheep. It is not infectious for man nor does it pass from sheep to goats. Transmission of sheep pox to goats is possible but exceedingly difficult according to Nocard.(5) It is not possible to immunize cattle against cowpox with sheep-pox virus. Sheep pox may then be regarded as an independent disease, although Gins and Rickert(6) consider all forms of pox as originating from human pox since they claimed to have changed human pox, swine pox, goat pox, and sheep pox into cowpox by passage through rabbits.

The virus of sheep pox.—The virus of sheep pox passes through Chamberland filters according to Borrel.(3) Definite formed elements have not been seen in the unfiltered lymph, though Borrel, Bosc,(7) and Paschen have described bodies similar to Guarnieri bodies in the cells of the purulent vesicular contents and in the epithelium of the skin. According to Bosc the virus may be present in the blood stream just before or at the beginning of the pox eruption. The virus is present in the scabs and after drying and becoming pulverized may be disseminated to healthy animals. In this way it is believed that the virus enters through the respiratory tract and penetrates the alveoli of the lungs to the blood stream by which it is distributed to the skin. Intravenous injections of lymph containing virus may result only in a febrile reaction without cutaneous eruption. Animals injected intracerebrally develop a febrile reaction six to eight days later and usually die within a week. Intracutaneous, subcutaneous, and intratracheal injections of sheep-pox virus in sheep are followed by the typical disease.

The virus of sheep pox in hermetically sealed tubes kept in a cool dark place will retain its virulence for two years or more. Stored at 35° C. it remains virulent for months. The virus in the fleece of sheep affected with the disease dies in at least two months. Presumably the virus is able to pass through the placenta, since affected ewes may transmit the disease to their offspring, the young being born with pox eruptions. The virus of sheep pox has not been cultivated.

Immunity in sheep pox.—One attack of sheep pox usually protects animals against this disease for the remainder of their lives. It is well known that young lambs are more susceptible than older sheep and usually become infected if the disease is prevalent in the herd. For this reason if the disease is prevalent at all it is preferable to induce an infection artificially than run the risk of natural infection which experience has shown is far more serious. Vaccination then becomes an emergency measure rather than a protective or preventive procedure. The method used to produce artificial infection employs the original virus and not an attenuated form, and practically amounts to inoculation such as was practiced in smallpox in olden times. The great danger of this method is the risk of spreading the disease among other healthy sheep that are not immune. This is particularly true since vaccination is carried on only in certain localities. Such immunization results in a lasting immunity, and the disease usually runs a very mild course. Freshly collected pox lymph may be stored in capillary tubes at low temperatures for considerable periods without losing its virulence. Bosc states that the virus of sheep pox remains alive and unattenuated for two years in the intestinal canal of leeches that have sucked lymph from ripe pox pustules.

Borrel has suggested a simultaneous method of immunization for producing lasting immunity. This method consists of one injection of 5 to 15 cubic centimeters of Borrel's immune serum, and at the same time elsewhere on the body 0.5 cubic centimeter of virulent lymph is given. This method of immunization has been used extensively with very excellent results.

Bridré and Boquet(8) have suggested a method of vaccination that employs sensitized pox lymph. The pox lymph is saturated with immune serum known to contain antibodies, and the high degree of immunity that results in sheep when treated with this vaccine is produced without producing pox lesions in the animal.

Complement-fixing bodies appear in the serum of sheep during the course of the disease and when used with an antigen prepared by extracting the pure pox scabs, complement is fixed. The serums of vaccinated animals also fix complement.

Pathological changes in sheep pox.—Animals dead of sheep pox show in addition to the skin lesions, hæmorrhagic inflammation of the membranes of the respiratory and gastrointestinal tracts. The lungs may show small nodules that have a central area of caseation necrosis. Other changes noted are infarcts in the lungs, acute interstitial nephritis, acute swelling of the lymph glands, and parenchymatous and fatty degeneration of the organs.

Control of sheep pox.—The most useful control measures for this disease are quarantine, reporting of outbreaks, proper disposal of infected carcasses, and vaccination of healthy animals in infected herds.

BIBLIOGRAPHY

1. Borgelat (1763). Quoted from Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Am. ed. by Mohler and Eichhorn. Alexander Eger, Chicago (1926) 374.
2. Chauveau, Journ. vét. (1868) 548.
3. Borrel, Soc. Biol. (1902) 59; A. P. **16** (1903) 123; Annal. de l'Inst. Pasteur (1903). Etude expérmentale de la clavelée.
   Borrel and Konew, Cbl. f. Bakt. (1907); Foth. Diss. Leipsig (1907).
4. Joest, Lit. on the last pox invasion in Germany, Zeit. f. Infkr. **1** (1906) 221.
5. Nocard, Rec. (1888) 272; Bull. (1898) 43 and 331; Bull. (1899) 263; **21** (1900).
6. Gins and Rickert. Quoted from Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Am. ed. by Mohler and Eichhorn. Alexander Eger, Chicago (1926) 374.
7. Bosc, Cbl. f. Bakt. **34** (1903) 413; Rev. gén. **4** (1904) 273.
8. Bridré and Boquet, C. R. **154** (1912) 144 and 1256.

VARIOLA EQUINA: HORSEPOX

SORE HEELS; PFERDEPOCKEN (GERMAN); VARIOLE EQUINE (FRENCH)
VAJUOLO EQUINO (ITALIAN)

Definition.—Horsepox is an acute, febrile, infectious disease of horses characterized by a vesicular exanthema appearing particularly in the flexor region of the pastern of young animals. In exceptional cases lesions also develop around the mouth and nose and on mucous membranes.

History.—Horsepox occurred most frequently during the middle of the last century, though at present its occurrence is rare and some authors, Dieckerhoff,(1) doubt its occurence entirely. Bouley,(2) Chauveau,(3) and Nocard and Laclainche(4) incline to take the view that so-called horsepox is the same condition as contagious pustular stomatitis and other vesicular eruptions that occur upon the genital organs of horses. In Germany these diseases are considered as independent conditions. While the matter is not settled and the close similarity of the three diseases must be admitted, horsepox may, for the time being, be considered a disease entity. Should it prove to be an independent disease such as pox in other animals, it may be assumed that, like other forms of pox, it owes its origin to the ancient occurrence of pox in the Far East.

Distribution.—The disease known as horsepox occurred in epizoötics during the nineteenth century in England, France, and Germany. Other diseases with which the diagnosis is often confused, such as the vesicular and pustular eruptions, have been more general in their distribution; and should horsepox eventually be identified with these conditions, its distribution will include a much wider area than it is at present considered to include. Vesicular eruptions have been described in South Africa, the United States, France, England, and Italy. Contagious pustular stomatitis and vesicular stomatitis will be considered later in detail.

Incubation period.—The incubation period in horsepox is usually considered to be from five to eight days.

Symptoms.—It is thought that the disease may be transmitted to horses by drivers and blacksmiths. Associated with the vesicular lesions that develop upon the skin of the pastern is a rise in temperature and functional disturbances of the extremities. The skin on the pastern is inflamed and swollen, and the vesicles develop to the size of lentils after which their contents exude and scabs are formed. In rare cases lesions develop on the mucous membranes of the mouth and nose and have been described on the conjunctiva of the eye.

Animals susceptible to the virus of horsepox.—The disease is usually limited to horses. However, Cameron(5) in 1908 reported a case of pox in man which occurred on the arm of a driver who treated a horse having horsepox. Chauveau succeeded in transmitting the disease to horses by injecting vaccine virus into the lymph and blood vessels. De Jong inoculated vaccine virus upon the mucous membranes of horses and produced lesions similar to those of pustular stomatitis and found that animals that had previously had this disease were immune to vaccine virus. This author also found that the stomatitis virus produced lesions in calves, rabbits, and cattle and Guarnieri bodies were demonstrated in the cornea of rabbits.

The virus of horsepox.—Jenner(6) first described pox disease in horses and attempted to find the source of cowpox in the pox eruptions of horses. Little investigative work has been done on the virus of this disease though it is known to be present in the vesicles that occur on the skin and in the saliva when the eruption involves the mucous membrane of the mouth. Such material when inoculated into the skin of cattle produces typical pox lesions. There are no data upon its filterability, though from what is known regarding other pox viruses the probability is that it will pass through coarse filters under high pressure.

Immunity in horsepox.—One attack of the disease produces immunity to future infection.

Control measures.—Isolation is the chief method of controlling and preventing the spread of the disease. If lesions are present on the mucous membranes of the mouth special pails for feeding and watering should be employed. Inoculation of healthy animals may be undertaken in order to shorten the course of the disease should it spread.

BIBLIOGRAPHY

1. DIECKERHOFF, Spez. Path. 1 (1892) 999.
2. BOULEY, Dict. de méd. vét. 9 (1871) 451.
3. CHAVEAU, Rec. (1866) 305, 625.
4. NOCARD and LACLAINCHE, Maladies microb. des animaux 1 (1903) 598.
5. CAMERON, Brit. Med. Journ. 1 (1908) 1292.
6. JENNER, S. P. 318.

For other references see Smallpox.

VARIOLA CAPRINA: GOAT POX

Definition.—Goat pox is an acute, febrile, infectious disease of goats characterized by a vesicular exanthema with moderate catarrhal symptoms involving the mucous membranes of the eyes and the respiratory passages.

History.—The origin of this disease, like other forms of pox, was most probably in Asia, and as other pox diseases it spread from India to Europe and Africa.

Distribution of goat pox.—The chief distribution of this disease has been in Norway according to Boeck(1) and Hansen.(2) It has also occurred in Italy, Spain, France, Germany, and

Algeria. A poxlike condition in goats has been reported in California.

Incubation period in goat pox.—The incubation period in goat pox is essentially the same as in sheep pox, six to eight days, but may vary in epidemics in different localities.

Symptoms.—The clinical course in goat pox is similar to the development of pox disease in sheep. The catarrhal symptoms are practically identical but perhaps not as severe in goat pox as in sheep pox. The development of the eruption is the same as in sheep pox. In goat pox abscesses may develop on the udder, and pox lesions may appear in the mouth and on the mucous membranes of the upper respiratory passages. In some cases warty granulations occur on the lips and nose and ulceration of the gums may occur. Usually the disease runs a favorable course, but it may begin with marked symptoms and terminate fatally. Complications such as pneumonia may alter the usual favorable course of the disease and result in the death of the animal.

Abortive forms of the disease have occurred in West Africa in which nodules only develop on the udder and teats which usually heal spontaneously within a short time. Similarly, a mild infection manifested by the appearance of hard nodules that simulate early lesions of goat pox has been described in California. Recovery from these mild forms of pox apparently does not produce immunity. While goat pox may be confused with foot-and-mouth disease, the differential diagnosis may be made on the facts that the disease is not transmissible to other cloven-footed animals and no lesions appear on the feet.

Animals susceptible to the virus of goat pox.—The virus of goat pox is not transmissible to sheep either by artificial inoculation or by natural infection. It does not pass to any other species of animals. If, as is supposed, the virus originated from cowpox and sheep pox, it is so changed in the goat that it is no longer infective for these animals. Ovine pox virus is said to be infective for goats by artificial inoculation, but Bonvicini(3) and Marcone(4) have never observed the disease in sheep in areas where goats were infected with it.

The virus of goat pox.—The virus of goat pox is present in the contents of the vesicles on the skin, and in the saliva if lesions have occurred in the mouth. Artificial infection may be produced in goats by inoculating them with such material. According to Marcone, small vesicles may develop on the hands and arms of humans who work around infected goats. Few investigations have been made with the virus of goat pox, but it is believed that the same general properties are possessed by this virus as are possessed by virus of sheep pox and cowpox.

Immunity in goat pox.—One attack of the disease produces a lasting immunity in goats. As has already been mentioned the mild forms of the disease produce little or no immunity. Preventive vaccination is possible but is little practiced.

Control of goat pox.—The general principles of control that have been described for sheep pox also hold for this disease.

BIBLIOGRAPHY

1. BOECK, D. Z. f. Tm. 9 (1879) 298.
2. HANSEN, Ref. Rep. (1890) 135.
3. BONVICINI, Il nuovo Ercolani (1898) 216.
4. MARCONE, La Rif. vet. (1900) 387.

OTHER REFERENCES

HERTWIG, Mag. (1840) 339.
GARBUTI and REALI, Clin. vet. (1905) 234.
VOIGT, A. f. Tk. 35 (1909) 204.
See also Smallpox.

VARIOLA SUILLA: SWINE POX

Definition.—Swine pox is an acute, febrile, infectious disease of swine, characterized by a papulo-vesiculo-pustular eruption that is quite generalized over the surface of the body, and is often accompanied with catarrhal symptoms especially involving the conjunctiva.

History.—Swine pox is rare but has appeared (1907) in Hungary, Roumania (according to Poenaru(1)), and Morocco. In former times sporadic cases have appeared in other southern and eastern European countries.

Course of the disease.—Spinola(2) and Szanto(3) describe swine pox as occurring especially in young pigs. The disease commences several days after infection with prodromal symptoms consisting of a rise in temperature, loss of appetite, stiff gait, chills, weakness, and a marked evaporation of moisture from the skin that results in a "greasy" appearance of the animals with the bristles standing on end. Early catarrhal symptoms develop in the form of a conjunctivitis. Small red spots appear on the eyelids, snout, abdomen, thighs, neck, and back, which soon develop into hard nodules. After two or three days vesicles develop, later these become purulent, then dry and form crusts and scabs. In very severe cases the vesicles may become coalescent and may be hæmorrhagic, and lesions may appear in the mouth, pharynx, trachea, and bronchi. Diarrhœa sets in, and death may result from exhaustion or complications such as pneumonia. The diagnosis of swine pox can be readily made by inoculating the contents of one of the vesicles on the skin ot a healthy animal.

The virus of swine pox.—Koch(4) reported the transmission of the virus of swine pox to calves, and it has long been known that cowpox virus can be transmitted to hogs. Szanto succeeded in transmitting swine pox to healthy pigs by placing infected pigs in the same pen with healthy ones. The incubation period was only four days. Poenaru transmitted the disease to healthy pigs with blood from infected hogs. It is thought that originally the disease developed in swine from cowpox, smallpox, and perhaps goat pox.

Immunity in swine pox.—Swine may be protected from swine pox by vaccination. The usual procedure and the one recommended is to inoculate the young healthy pigs with the contents of the ripe vesicle but not from pustules. Vaccination according to this method results in immunity, and the operation is carried out by the usual methods. While the mortality is usually not high in the natural infection, there has been ample opportunity to observe that recovered animals remain immune to subsequent infection with the virus.

BIBLIOGRAPHY

1. POENARU, Arch. vet. (1907) 67.
2. SPINOLA, Krankheiten d. Schweine (1892) 204.
3. SZANTO, A. L. (1906) 541.
4. KOCH, O. M. (1908) 1887.

For other references see Smallpox.

ILLUSTRATIONS

PLATE 1

FIG. 1. Smallpox and varicella. (From Hartzell; after Pfaundler and Schlossman.)

2. Vaccination with vaccine virus. Eight days after vaccination. Vaccine virus twenty-seven days old. (Courtesy of Dr. Otto Schöbl.)

PLATE 2

Generalized vaccinia in man. (Courtesy of Dr. Otto Schöbl.)

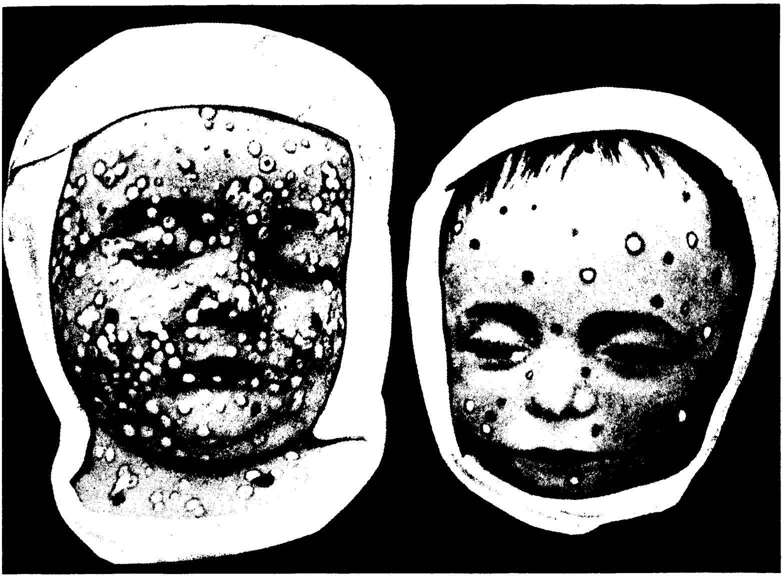
PLATE 3

Alastrim. Patches on inner side of thigh, above knee and around septic cut. (After Moody, from Manson's Tropical Diseases.)

PLATE 4

FIG. 1. Cowpox on the udder. (After Fréger.)

2. Sheep pox. (After Joest.)



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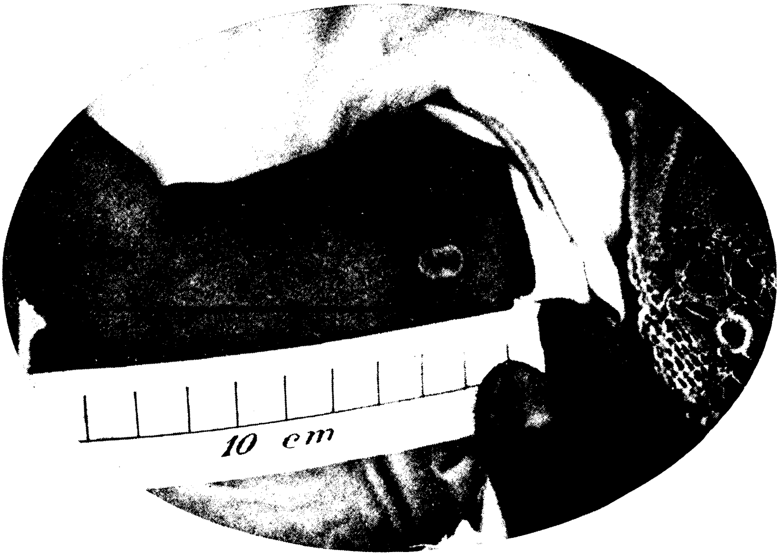

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Fig. 1. Smallpox and varicella. 2. Vaccination with vaccine virus.

PLATE 1.

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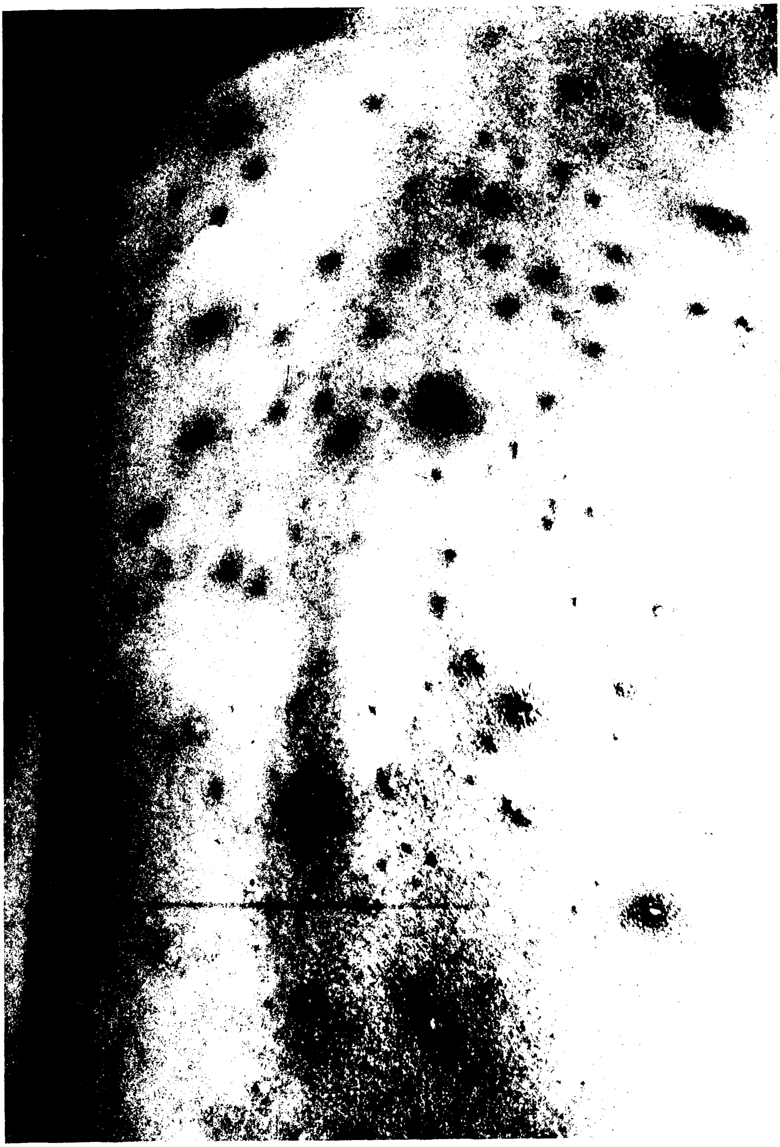

PLATE 2. GENERALIZED VACCINIA IN MAN.

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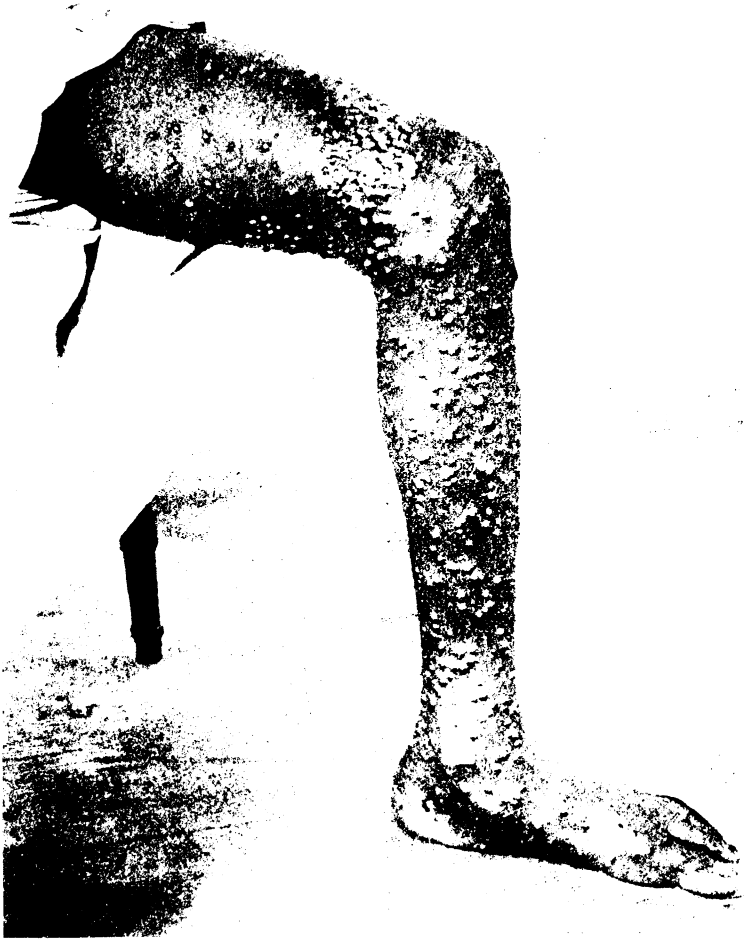

PLATE 3. ALASTRIM. PATCHES ON INNER SIDE OF THIGH, ABOVE KNEE AND AROUND SEPTIC CUT.

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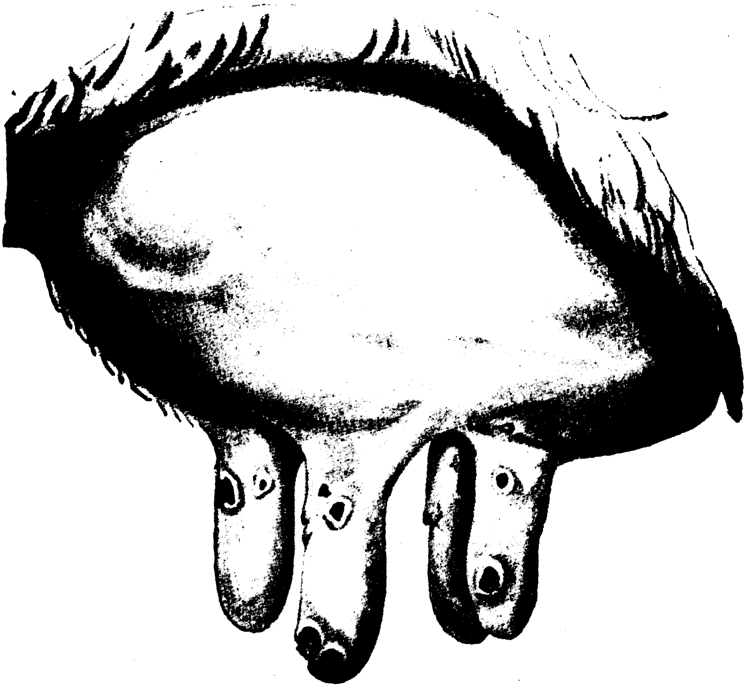

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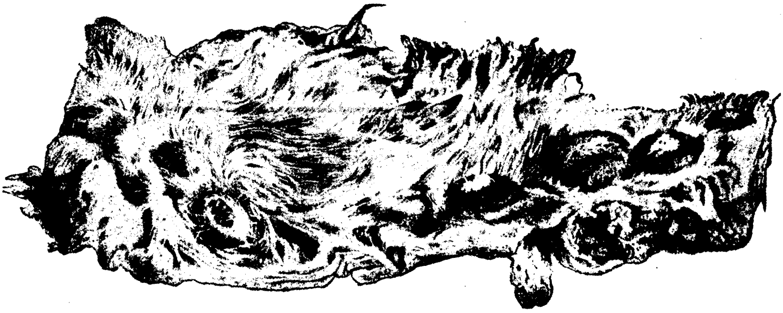

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Fig. 1. Cowpox on the udder. 2. Sheep pox.

PLATE 4.

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CHAPTER III

FILTERABLE VIRUS DISEASES OF MAN AND ANIMALS (CONTINUED)

VARICELLA: CHICKEN POX

VARIOLETTE (FRENCH); WASSERPOCKEN (GERMAN); MOROIGLIONE (ITALIAN)

Definition.—Varicella is a markedly contagious disease, characterized by a cutaneous eruption that is confined to the superficial layers of the skin. Chicken pox is usually a harmless disease and is rarely malignant. Death never results from this disease, and as a rule there are only mild constitutional symptoms associated with it. The exact cause of chicken pox is unknown, but it is thought to be caused by a filterable virus.

History.—Undoubtedly chicken pox has existed since ancient times, though it was confused with smallpox (see Chapter II) until the nineteenth century. By most of the early physicians this disease was regarded as a benign form of smallpox. Chicken pox has always been a mild disease, but it was early recognized to be highly contagious and is one of the most important diseases of children from the standpoint of loss of time from schools, and is an important consideration in institutions such as hospitals for children. We are informed by Vaughan that even during the latter half of the nineteenth century Hebra, of the Department of Dermatology in the University of Vienna, taught that chicken pox is a mild form of smallpox. There is no question as to the relation of chicken pox to smallpox since it is now well known that neither condition will give any immunity against the other, although an attack of either disease confers an immunity against a subsequent attack of the same disease. Chicken pox is found everywhere. No age is exempt, although the majority of cases occur in the fifth or sixth year. It has been estimated that 52 per cent of adults have had the disease during childhood. The disease is more prevalent in girls than in boys, and among white children than among colored children.

The virus of chicken pox.—Little is known concerning the virus causing this disease. Numerous attempts have been made to infect human beings artificially with varicella material and to transmit the disease to laboratory animals. Bryce,(1) in 1816, inoculated vesicular material taken from cases of chicken pox, representing all stages of the disease, into the skin of children who had never had smallpox or chicken pox. None of the children developed lesions. Hesse inoculated one hundred thirteen children with varicellous material and in seventeen of them obtained a slight local reaction, while nine developed a general reaction. Other investigators have had more or less success, but in general, the reports dealing with experimental chicken pox are at most unsatisfactory. In animals practically no success has been met with. Rivers and Tillett(2) have succeeded in inducing lesions in rabbits and monkeys with material taken from chicken-pox vesicles, a description of which will be given under Virus III infection. The possible relation of varicella to herpes zoster has been the subject of numerous papers during the last few years. Le Feuvre(3) is of the opinion that these two diseases are related etiologically. Suggestive data in this connection are contained in the work of Lauda and Silberstern,(4) Meldrum,(5) Matthews,(6) Pereira,(7) and others. Ziel(8) also speaks of a "zosteriform" chicken pox; Cozzolino,(9) Hoffmann,(10) and Netter and Urbain(11) have reported work which suggests the identity of the virus of herpes zoster and varicella. Recently, however, Scheer(12) reports herpes zoster and coincident varicella in a brother and sister developing simultaneously, one zoster and the other varicella. Fourteen days later, the child having zoster developed varicella and Scheer concludes that zoster offers no protection to chicken pox.

In the differential diagnosis of this disease the physician has been concerned chiefly with smallpox. Histologically, smallpox vesicles are found to contain so-called vaccine bodies, while chicken pox vesicles do not contain these. The lesions produced upon the rabbit's cornea with vaccine virus contain vaccine bodies, while the lesions produced with chicken pox are very minor and do not contain inclusion bodies.

Though the virus of chicken pox has not been discovered we may state definitely that it is not related to the virus of small pox and probably is unrelated to the virus of herpes zoster.

Incubation period.—The incubation period of chicken pox usually ranges from fourteen to twenty-one days. The period of incubation varies from four to twenty-one days but for public health purposes twenty-one days is considered the maximum.

Symptoms.—Mild prodromal symptoms consisting of general malaise may precede the eruption. Usually the first symptom of the disease is the eruption. In rare cases a scarlatiniform erythema is noted preceding the rash.(13) The eruptions begin first upon the head and trunk, then extend to the extremities. They appear in successive crops over several days. The trunk is usually more extensively involved. Lesions usually appear upon the face and in some cases may be numerous in this locality. With the eruption there is usually a slight rise in temperature to 99 or 100° F. The beginning lesion is a small erythematous spot which gradually develops into a vesicle containing a clear fluid. The vesicles are small, pinhead in size to pea size, round or oval, very superficial and thin walled. In some cases the contents of the vesicle may become purulent. After one to three days the lesion dries up and a thin yellowish crust is formed. The lesions are always discrete and irregular in distribution. Several stages of development of the lesions may be noted at a given time. The mucous membranes are usually attacked, a few lesions occurring on the hard and soft palate. Itching may or may not be a prominent symptom. The disease may be complicated by secondary infection, and measures should be instituted to avoid this.

Immunity.—One attack of chicken pox confers a definite immunity. A number of investigators have attempted to induce artificial immunity in susceptible children by vaccination with the contents from varicella vesicles. In 1913, Kling(14) obtained success with this procedure. This method of inducing artificial immunity has also met with more or less favorable results in the hands of Handrick,(15) Rabinoff,(16) Michael,(17) and Greenthal.(18) The latter in 1926 obtained nineteen "takes" among thirty-six persons vaccinated with the vesicular fluid of chickenpox lesions. Greenthal concludes that this method of producing immunity serves to check the spread of the disease. In his hands the immunity lasted less than eighteen months in one instance and more than six weeks in another. Greenthal attempted to inoculate guinea pigs with varicellous material by the coal-tar method employed by Teague and Goodpasture(19) in herpes zoster, but his experiments were unsuccessful.

Pathology.—The vesicle of chicken pox is multilocular. According to Unna its cavities are formed by the reticulating liquefaction of groups of prickle cells in the middle and upper portion of the rete. Ormsby states that the septa are formed of cells between the cavities that have not undergone liquefaction. The contents of the vesicle are said to consist of granular coagulated fibrin and ballooned cells. The corium shows œdema and vascular dilatation, but practically no infiltration of leucocytes.

Prevention.—Cases of chicken pox should be isolated. Kling favors vaccination with chicken-pox virus during epidemic conditions. Greenthal's work also suggests the advisability of this. Hess and Unger (20) were able to obtain immunity to varicella in thirty-seven of thirty-eight cases following the intravenous injection of vesicular fluid. This method of immunization should receive further attention, and the duration of such induced immunity should be accurately determined.

HERPES ZOSTER: SHINGLES

ZOSTER, ZONA; GÜRTELFLECHTE (GERMAN)

Definition.—Herpes zoster is an acute disease characterized by the appearance of groups of vesicles, usually following the course of a nerve trunk, and accompanied by neuralgic pains or itching. The distribution of the lesions is usually unilateral, and generally is not recurrent.

Symptoms.—Hyperæsthesia, itching, or pain may precede the appearance of the herpetic lesions of zoster. The pain may be localized or extend beyond the site of the future eruption and in some cases the glands near the site of the eruption become enlarged and painful. The vesicles appear along the course of the affected nerve and tend to occur in groups of two, three, or more, in patches separated by normal skin. The vesicles, which may be the size of a large pea and contain a clear fluid, later develop into pustules. In some instances groups of vesicles coalesce and are deeply situated in the skin. The lesions develop in successive groups and finally dry up and form a yellowish crust that leaves a pigmented area on the skin. In few cases ulceration may take place. Usually the disease runs its course within two to three weeks but may be prolonged in some cases. The pain in herpes zoster may be very mild or severe depending upon the case, and may be transitory, disappearing with the lesions, or persist for long periods of time after all symptoms of the disease have subsided. While the disease is not prone to recur, many cases of two or more attacks are on record. The eruption may occur in different localities, and upon the basis of its place of attack the types of the disease have been designated. Dermatologists now recognize the following types of zoster: Zoster frontalis, ophthalmicus, capillitii, facialis, nuchæ, brachialis, pectoralis, abdominalis, and femoralis.

The virus of zoster.—The true etiologic agent of herpes zoster is unknown. It is thought that infection, and mechanical and chemical trauma are its chief causes. Rosenow and Oftedal(21) have reported the experimental production of zoster lesions in animals following the injection of certain streptococci which they isolated from the tonsils and pyorrheal pockets of patients suffering with zoster. Sachs(22) has described an epidemic of some sixty-nine cases of the disease that occurred in Breslau in 1901 which indicates the infectious nature of the malady. Hay(23) is of the opinion that the enlargement of the lymph glands in zoster indicates its infectious character. The relation of herpes zoster to varicella has been mentioned above. The affection has also been noted in connection with focal infections by Lain,(24) with malaria by Winfield,(25) with syphilis by Brown and Dujardin;(26) and by other authors it has been noted with eclampsia, pyæmia, septicæmia, meningitis, manipulations of the spine, gunshot wounds, and other injuries, and following the extraction of teeth. Also it appears that this infection may follow the ingestion of arsenic, and poisoning with monoxide, carbon dioxide, and other substances.

In connection with the possible relation of herpes zoster to chicken pox Greenthal(18) states that while working with the virus of chicken pox he developed a mild herpes zoster. He had had chicken pox during childhood. Recently one of my patients developed a mild herpes zoster although he had suffered an attack of chicken pox only a year before. Examples such as these can be multiplied. We believe that herpes zoster and chicken pox are independent diseases and are caused by different agents. These agents may be closely related, but they appear to be specific.

Lipschütz(27) reports that he has been able to demonstrate the presence of inclusion bodies in the lesions of zoster that are similar to the bodies found in other forms of herpes and in many other diseases that are caused by filterable viruses. From the vesicles of herpes simplex and herpes genitalis a virus can be recovered that is infective for rabbits, producing in them an encephalitis to which they succumb within a few days. The virus can be recovered from the brain of the infected animal. Cole and Kuttner(28) in 1925 examined nine cases of herpes zoster and attempted to infect rabbits with fluid from the vesicular lesions. These authors were unable to isolate a single strain of herpes from these cases. During the following year McKinley and Holden (29) in their studies on experimental encephalitis in rabbits attempted to isolate a strain of herpes from three cases of herpes zoster but were unsuccessful. It seems apparent that the virus of herpes zoster is not infective for rabbits at least when it is inoculated subdurally. As mentioned above, Teague and Goodpasture (19) were able to inoculate guinea pigs with herpes virus quite easily after the skin had been rendered susceptible with previous coal-tar applications; in this way they produced experimental herpes zoster.

The evidence points to a filterable virus as the etiologic agent in herpes zoster. Mechanical and chemical traumatism may be factors assisting in the development of the infection. As we pointed out in another publication (30) the herpes virus may remain latent in the tissues (ganglia, nerve trunks, etc.) and pass down the nerve trunks under certain favorable conditions and manifest itself in a cutaneous lesion.

Incubation period.—The exact period of incubation in herpes zoster is unknown.

Animals susceptible.—Man is the natural host for the virus of herpes zoster. According to the experiments of Goodpasture and Teague guinea pigs under certain conditions are also susceptible.

Immunity.—Herpes zoster is not prone to recur. However, instances in which several attacks of the disease have occurred in the same patient are on record. These observations may be taken as both positive and negative evidence for immunity. At present the question remains unsettled. Herpes zoster does not protect against chicken pox nor does an attack of chicken pox protect against herpes zoster. Chicken pox, however, confers a definite immunity against a subsequent attack of varicella. On these facts alone should be based the opinion that these two conditions are separate and distinct entities although they may be similar and the causative agents may be closely related.

Pathology.—The chief changes in herpes zoster are found in the posterior roots of the spinal nerves, in the root fibers of the posterior columns of the cord, and in the peripheral nerves. These changes consist of interstitial inflammation and degenerative changes of the ganglionic centers. Sunde (31) found active inflammation, multiple small hæmorrhages, and round-cell infiltration in and around the Gasserian ganglion on the affected side of a patient having herpes zoster frontalis. There was also a round-cell infiltration between the nerve fibers and a fibrinopurulent exudate associated with the presence of a Gram positive (both diplococci and chains) coccus. Regeneration usually takes place, but in some cases the destruction is complete and permanent. The cutaneous changes involve both the epidermis and the corium. Unna describes the changes in the epithelial cell as a "ballooning degeneration." The cells within and around the vesicles have a cloudy protoplasm and frequently contain a number of nuclei which resemble protozoans. These were once thought to be parasites, but it is now known that they are nuclei. In the corium there is also dilatation of the capillaries, œdema, and moderate leucocytic infiltration.

Herpes zoster is a self-limited disease and usually terminates within a few weeks at most. Herpes simplex tends to recur, and its appearance is not related to the nerve distribution. Herpes zoster should be differentiated from eczema and from chicken pox, which in some cases closely resemble it clinically.

VIRUS-III INFECTION

Virus III was discovered by Rivers and Tillett(2) while attempting to produce chicken pox in rabbits. The agent produces gross as well as microscopic lesions in the cornea, skin, and testicles of rabbits, and infection with this virus results in an immunity against subsequent infection with the same material. In the beginning Rivers and Tillett believed that this virus was the cause of chicken pox, but further work disclosed the fact that virus III is indigenous to rabbits and that it can be differentiated from vaccine virus as well as herpes virus. These authors demonstrated that the localization of the virus of varicella in the human skin is influenced by irritation. The effect was observed in five of fifty-one patients. In one case the lesions beneath an adhesive-plaster irritant were found to be more numerous than on other parts of the body. In another case napkins were irritants, and the lesions of chicken pox were located at the site of the irritation. In another case the tie and collar served as an irritant. This suggests that the virus is in the circulating blood. Rabbits were found to be susceptible to the virus recovered from the blood of varicella patients, which when inoculated into the testicles of rabbits produced redness, swelling, and œdema, although no ordinary bacteria could be demonstrated. The virus was capable of serial transmission through rabbits by means of testicular inoculation and resisted the action of 50 per cent glycerol for twenty-nine days.

Andrews and Miller,(32) and Miller, Andrews, and Swift(33) have also described a filterable virus infection of rabbits occurring in apparently normal rabbits and in animals inoculated with rheumatic-fever material. It is quite probable that these viruses, if not identical with virus III of Rivers and Tillett, are closely related to it.

In later experiments Rivers and Tillett demonstrated that virus III is filterable through Berkefeld N and V candles, and that the virus is destroyed by heating to 55° C. for ten minutes. These authors found that the intradermal inoculation of virus III into rabbits results in more-uniform lesions than those obtained by smearing the virus on the scarified skin. Immunity to virus III was produced by intradermal, intratesticular, intravenous, intracerebral, and intranasal inoculations. This immunity persists for at least six months. Passive immunity could not be produced in normal rabbits by immune serum taken from immunized animals. Serum from immunized animals, however, possesses virucidal properties for virus III either in vitro or when injected simultaneously with the virus into the same part of the skin. Rivers and Tillett state that virus III is immunologically distinct from both vaccine virus and the virus of symptomatic herpes. Serum from several patients convalescing from chicken pox possessed no virucidal activity against virus III. The authors conclude that the experimental evidence shows no relation of the virus to that of varicella.

In 1926 Rivers,(33) convinced that virus III was indigenous to rabbits and that it was not related to chicken pox, reported his experiments with varicella material inoculated into monkeys (vervets). In this work he was able to demonstrate that following intratesticular inoculation of varicella material into monkeys there appeared nuclear inclusions characteristic of those found in several other filterable virus diseases. These inclusions were observed in the glandular cells of the testicle removed on the sixth day and stained with eosin. In a second monkey showing a similar gross reaction to the inoculation the testicles were removed on the eighth day but no inclusions were found. These results offer encouragement for the future study of chicken pox and should receive further attention.

Virus III has apparently been excellent material with which to study some of the more fundamental problems in relation to the general problem of the filterable viruses. Many interesting experiments suggest themselves with material at hand such as this. Pearce and Rivers have carried on some interesting work with this virus in connection with tumor growth and malignancy. These authors found that transplantable rabbit neoplasm does not develop in animals immunized to virus III as it does in nonimmunized animals. They believe that this resistance to the neoplasm is nonspecific and is due to a more effective resistance of the host. In other experiments these authors were able to show that animals inoculated with virus-III-infected tumor material developed a more serious disease than animals receiving non-virus-bearing tumor material.

Virus III should receive further study in an effort to confirm the interesting results that have been obtained by Rivers and his associates and to extend as far as possible our fundamental knowledge concerning the filterable virus infections.

BIBLIOGRAPHY

1. BRYCE (1816). Quoted from Vaughan's Epidemiology and Public Health. C. V. Mosby & Co., St. Louis 1 (1922) 213.
2. RIVERS and TILLETT, Journ. Exp. Med. **38** (1923) 673; **39** (1924) 777; **40**: 281.
   RIVERS and PEARCE, Journ. Exp. Med. **42** (1925) 523.
   PEARCE and RIVERS, Journ. Exp. Med. **46** (1927) 65, 81.
3. LE FEUVRE, Brit. Journ. Derm. **29** (1917) 253.
4. LAUDA and SILBERSTERN, Med. Klin. **22** (1926) 374.
5. MELDRUM, Brit. Med. Journ. **2** (1926) 302.
6. MATTHEWS, Brit. Med. Journ. **2** (1926) 835.
7. PEREIRA, Brit. Med. Journ. **2** (1926) 597.
8. ZIEL, Med. Klin. **22** (1926) 991.
9. COZZOLINO, Pediatria **34** (1926) 809.
10. HOFFMANN, Deutsch. med. Woch. **52** (1926) 864.
11. NETTER and URBAIN, Compt. Rend. Soc. de Biol. **90** (1924) 461.
12. SCHEER, Jahrbuch f. Kinderheilkunde, Berlin **117** (1927) 343.
13. ORMSBY, Disease of the Skin. Lee and Febiger, Philadelphia (1927).
14. KLING, Berl. klin. Wchnschr. **50** (1913) 2083.
15. HANDRICK, Monatschr. f. Kinderh. **13** (1914) 242.
16. RBINOFF, Arch. Pediat. **32** (1915) 651.
17. MICHAEL, Arch. Pediat. **34** (1917) 702.
18. GREENTHAL, Am. Journ. Dis. Children **31** (1926) 851.
19. TEAGUE and GOODPASTURE, Journ. Am. Med. Assoc. **81** (1923) 377.
20. HESS and UNGER, Am. Journ. Dis. Children **16** (1918) 34.
21. ROSENOW and OFTEDAL, Journ. Am. Med. Assoc. (1915) 1968.
22. SACHS (1906) F. 12, V. 25; Rev. Prat. d. Med. Cut. Syph. et Ven., Nos. 1 and 2 (1907) 9 and 219.
23. HAY, Journ. Cut. Dis. **16** (1898) 1.
24. LAIN, Journ. Cut. Dis. **35** (1917) 486.
25. WINFELD, N. Y. Med. Journ. **76** (1902) 191.
26. BROWN and DUJARDIN, Brain, London **42** (1919) 86.
27. LIPSCHÜTZ, Arch. f. Dermat. u. Syph. **126** (1921) 428.
28. COLE and KUTTNER, Journ. Exp. Med. **42** (1925) 799.

29. McKinley and Holden, Journ. Infect. Dis. 39 (1926) 441.
30. McKinley and Holden, Arch. Path. and Lab. Med. 4 (1927) 155.
31. Sunde, Deutsch. med. Wchnschr. 18 (1913) 849.
32. Andrews and Miller, Journ. Exp. Med. 40 (1924) 789.
33. Miller, Andrews, and Swift, Journ. Exp. Med. 40 (1924) 773.
34. Rivers, Journ. Exp. Med. 43 (1926) 275.

RUBEOLA: MORBILLI: MEASLES

Definition.—Measles is a specific, highly contagious, febrile disease which is characterized by catarrhal manifestations of the upper respiratory tract, suffusion of the eyes, and a definite cutaneous rash. While the etiology of measles is not definitely determined it is thought by some to be caused by a filterable virus and by other investigators to be of definite microbial origin.

History.—Measles is one of the most readily communicable of all diseases. Man is universally susceptible. It is probable that this disease is as old as the human race though in olden times it was confused with smallpox. Sydenham (1) in 1847 first gave an accurate description of this disease which eliminated much of the confusion between measles and smallpox that had gone before. Later, for a time, measles was confused with scarlet fever, and even to-day there are cases of German measles that offer difficulty in arriving at an early diagnosis between these two diseases. Koplik in 1896 described small, irregular, red spots, the centers of which are the seat of minute bluish-white specks, on the mucous membranes of measles cases. These spots, now designated "Koplik spots," appear before the cutaneous eruption and aid materially in the diagnosis of this disease.

While measles is considered one of the commonest diseases of childhood, it is by no means limited to this age group. According to Vaughan (2) there were 67,763 cases of measles with 4,264 deaths among the soldiers of the Union Army during the Civil War, while during the World War in the year 1918 the Surgeon General's report gives 48,900 cases of measles for the United States Army. Pneumonia is a frequent complication of this disease, and the relatively high mortality is due to this complication. Wherever people are brought together in crowds, and particularly in time of war, there are susceptible individuals, and epidemics of measles are to be expected.

Distribution of measles.—Man is universally susceptible to measles, and the distribution of this disease is limited only by the distribution of its natural host.

The virus of measles.—The specific etiological agent in measles is undetermined. It is known from the work of Goldberger and Anderson that the virus of measles is present in the secretions from the nose and throat of measles cases, and that the specific agent is capable of passing through the pores of a Berkefeld filter. Blake and Trask(3) were able to induce the disease in monkeys with material obtained from the nasopharynx of active cases, and further demonstrated that the virus is filterable. This virus resists drying and freezing for twenty-four hours but is destroyed at 55° C. Hektoen(4) produced measles experimentally in two students, while Anderson(5) has also succeeded in inducing the disease in monkeys. Nevin and Bittman(6) claim to have induced symptoms of the disease in guinea pigs following the injection of blood from active cases of the disease. Duval and d'Aunoy(7) have obtained similar results. None of these experiments has resulted in the isolation of any known bacterial forms, though Mallory has described certain minute bodies in the endothelial cells of the capillaries of measles cases.

Caronia(8) recently reported the presence of an organism consisting of very minute granules and occurring in pairs in the blood, bone marrow, cerebrospinal fluid, and filtrates from nasal secretions of cases early in the course of the disease. This organism may be cultivated anaërobically from material that has been passed through a Berkefeld filter. Further, according to this investigator, the disease may be produced in susceptible individuals following the inoculation of this culture intravenously and the killed culture when injected produces immunity to the disease.

More recently Tunnicliff,(9) Donges,(10) Ferry and Fisher,(11) and Hibbard and Duval(12) have cultivated a nonhæmolytic streptococcus from the blood of measles cases. Tunnicliff has also been able to cultivate another streptococcus from cases of German measles. In forty-two of fifty-two cases this author recovered a streptococcus, although in twenty cases other bacterial forms were found in addition to the streptococcus. Ferry and Fisher and also Tunnicliff have demonstrated that their streptococcus produces a toxin and in this respect is quite similar to the streptococcus of scarlet fever. In this connection Paraf(13) has shown that measles complicated by streptococcus infections can cause a positive Dick reaction to become negative. In later experiments Tunnicliff has shown that the green-producing diplococci isolated before the appearance of the rash and during the acute stages of the disease are immunologically distinct from similar cocci isolated later during the convalescence of the patient. Guardabassi(14) found from his experiments on rabbits and guinea pigs that the measles virus is filterable, and demonstrated Gram-negative granular formations that measure from 0.4 to 0.6 micron in length and stain pink with Giemsa's. He states that these forms are analogous to those described by Coronia.

Long and Cornwell(15) attempted to isolate a toxin-producing green streptococcus from forty-seven cases of measles but were unsuccessful. In all the cultures made from the blood of these patients during the preëruptive stage of the disease, cultures were obtained in only four instances and these were regarded as contamination.

Degkwitz(16) has been a steady proponent of the filterable virus theory of the etiology of this disease. In his most recent work he states that measles can be produced in human beings with material sterile from a bacteriologic standpoint, with sterile blood from a patient with measles, or with dilutions of blood that have been passed through a Berkefeld filter. This author finds that the nasal secretions are also infective. According to this author the virus of measles may remain alive for several weeks in blood taken from a patient having the disease, provided it is placed in buffered salt solution and kept at 0° C.

Other organisms have been described as the cause of this disease by Salimbeni and Kermorgant(17) who described the cultivation of a spirochæte associated with a Gram-negative bacillus from measles cases and by Sellards and Bigelow(18) who reported the discovery of a small Gram-positive bacillus. Kusama(19) has described the passage through monkeys of a Gram-positive diptheroid bacillus that he believes to be the cause of measles.

It may be said without reservation that the virus of measles is at present unknown. Since so many different organisms have been described as the cause of this disease it appears that secondary bacterial invasion in measles cases is apparently accomplished with great ease. Perhaps future investigations will determine which of the various organisms described is the true cause of this disease or whether we must continue to regard the etiological agent of measles as a filterable and invisible agent.

Incubation period in measles.—The incubation period of measles is usually from eleven to fourteen days.

Symptoms.—The symptoms of measles may be divided into three stages; namely, the period of invasion, the stage of eruption, and the stage of desquamation.

The invasion of measles is gradual. The fever and the catarrhal symptoms increase gradually until the rash appears. The invasion of the disease is characterized by coryza, increased lachrymation and suffusion of the eyes, photophobia, sneezing, and nasal discharge. A hoarse, hard cough usually develops, and the patient may develop a sore throat. On the mucous membrane of the cheeks appear minute white spots, Koplik spots, that are diagnostic of the disease before the rash appears. The constitutional symptoms consist of dullness, headache, pains in the back and legs, and in some cases vomiting and diarrhœa.

The eruption or rash begins about the third to the fifth day of the disease as small dark red spots on the back or behind the ears, at the hair line over the forehead, and on the neck. The first lesions are macules which rapidly change, within about twenty-four hours, to papules. The rash is usually fully developed in from thirty-six hours to three days. As the disease progresses the rash spreads over other parts of the body and appears last upon the lower extremities. As a rule it covers the entire body and may remain discrete or become confluent. Usually the rash lasts about four days, but in mild cases it may terminate within a day or two and in other cases remain for six days or a week. The constitutional symptoms reach their height at the time of the full development of the rash. The tongue becomes coated and somewhat resembles the strawberry tongue of scarlet fever. As the rash subsides the general symptoms become less marked, the fever rapidly declines and within twenty-four to forty-eight hours after the temperature has reached normal the rash disappears.

When the rash disappears a fine branlike desquamation begins. It may last from five days to two weeks and is more marked in the cases where the eruption has been severe.

Measles cases are subject to a variety of complications. As a rule the mortality is low, but in epidemics where the disease is complicated by a terminal pneumonia the mortality may be exceedingly high. In addition to pneumonia other complications, such as meningitis, encephalitis (see chapter on Encephalitis), otitis, chronic conjunctivitis, enlargement of the lymph glands followed by tuberculosis infection, nephritis, endocarditis and pericarditis, gastric disorders, erysipelas, furunculosis, impetigo, pemphibus, and hæmorrhages, have been observed. Measles may also be complicated by other infectious diseases.

The blood picture in measles is characterized in the early stages of the disease by a lymphocytic leucocytosis and later there is a leucopenia.

Animals susceptible to the measles virus.—Man is the natural host for this virus. Experimentally monkeys, rabbits, and guinea pigs are said to be susceptible.

Immunity.—One attack of measles usually confers immunity. Second attacks are more often reported in this disease than in any of the other eruptive fevers. Age is no barrier to the infection provided the individual has never suffered an attack of the disease. It is quite generally agreed that measles lowers the resistance to other infections more than any other disease. Immune bodies are greatly diminished or disappear within a short time after an attack of measles.

Within recent years the use of convalescent serum has been employed, particularly by Park, for the prevention of measles. The serum is most active after the tenth day following the disease and before three months have elapsed. Five cubic centimeters of this serum are sufficient to immunize a child against measles for a few weeks. Adult serum has also been employed for this purpose, but much larger quantities are required to produce any immunity. Debre, Joanmon, and Papp(20) have employed minute quantities, 0.00125 cubic centimeter, of filtered blood from measles cases for the purpose of immunizing children and state that hundreds of children have been successfully vaccinated in this way without any reactions. The duration of the immunity produced by this method is unknown. According to Baron(21) the use of Degkwitz's protective sheep serum for measles is not encouraging. In this author's experience all persons so immunized developed measles and in some cases with grave results. On the other hand the use of convalescent serum for immunization has met with continued success wherever it has been tried. For the time being this method of prevention in measles appears to be very encouraging, and the dangers of transmitting other infectious diseases may be minimized by careful selection of material to be used for immunization of susceptible individuals.

In 1926 Tunnicliff and Hoyne(23) reported the immunization of goats with their green-producing measles diplococci and found that the serum from these goats protected rabbits against subsequent injections of infective material from measles. Rabbits not so protected developed characteristics of measles when injected with infective material, the symptoms consisting of a rise in temperature, Koplik spots, and a rash. In another paper these authors have described the results of the use of their immune serum in children in which 45 per cent of children who received 4 to 6 cubic centimeters of serum on the fourth day after contact with measles patients remained immune. All infants under one year of age who received serum after the fourth day following exposure developed the disease. Tunnicliff concludes that—

> Although the duration of passive immunity with immune goat serum, as with human convalescent measles serum, is only a few weeks, the serum appears to be useful in preventing measles in very young and sick children, and in stopping epidemics in institutions where the inconvenience of an epidemic is great and the mortality may be high.

Pathology.—The only anatomical changes in uncomplicated measles are those found in the skin and mucous membranes. The skin lesions are inflammatory in character and are thought to be more superficial than those in scarlet fever. Around the blood vessels there is an infiltration of round cells. Œdema and congestion are in evidence about the sweat and sebaceous glands, and the papillæ.

The mucous membranes are the seat of a catarrhal inflammation, and in some cases the inflammation may be of a membranous character. Other anatomical changes depend upon various complications which may appear in certain percentages of measles cases.

Control measures.—Isolation and quarantine are absolutely essential for the control of this disease. Seroprophylaxis, as soon as it can be carried on on a large enough scale, may offer great aid in the prevention of this disease. At present our methods of preventing measles are totally inadequate, even were they rigidly enforced, due primarily to the very nature of the disease. Rosenau (22) states, "the suppression of measles is one of the most difficult problems we have to face, for the reason that the disease is one of the most highly contagious of all infections, and for the further reason that it is most contagious during the preëruptive stage." Further investigations it is hoped will lead to a better knowledge of this disease upon which a practical and definite method of protective immunization may be devised.

BIBLIOGRAPHY

1. Sydenham, Translation by Latham, London (1847). Quoted from Vaughan's Epidemiology and Public Health.
2. Vaughan, Epidemiology and Public Health. C. V. Mosby & Co., St. Louis (1922).
3. Blake and Trask, Journ. Exp. Med. **33** (1921) 385.
4. Hektoen, Journ. Infect. Dis. **2** (1905) 238.
5. Anderson and Goldberger, Pub. Health Rep. **26** (1911) 847, 887; Journ. Am. Med. Assoc. **6**: 971.
6. Nevin and Bittman, Journ. Infect. Dis. **29** (1921) 429.
7. Duval and d'Aunoy, Journ. Exp. Med. **35** (1922) 257.
8. Caronia, La Pediatria **31** (1923) 801.
9. Tunnicliff, Journ. Am. Med. Assoc. **68** (1917) 1028; **71** (1918) 104; Journ. Infect. Dis. **22** (1918) 462; **24** (1919) 76, 181; **37** (1925) 193; **41** (1927) 267.
   Tunnicliff and Brown, Journ. Infect. Dis. **23** (1918) 572.
   Tunnicliff and Moody, Journ. Infect. Dis. **31** (1922) 382.
   Tunnicliff and Hoyne, Journ. Infect. Dis. **38** (1926) 48.
   Tunnicliff and Taylor, Journ. Am. Med. Assoc. **87** (1926) 846.
10. Donges, Central. f. Bakt., Arb. I, Orig. **91** (1923) 45; **94** (1925) 115.
11. Ferry and Fisher, Journ. Am. Med. Assoc. **86** (1926) 932.
12. Hibbard and Duval, Proc. Soc. Exp. Biol. and Med. **23** (1926) 853.
13. Paraf, Bull. et mém. Soc. med. d. hop. de Paris **50** (1926) 506.
14. Guardabassi, La Pediatria **35** (1927) 801.
15. Long and Cornwell, Journ. Infect. Dis. **40** (1927) 408.
16. Degkwitz, Münch. med. Woch. **73** (1926) 181, 248.
    Journ. Infect. Dis. **41** (1927) 304.
17. Salimbeni and Kermorgant, Compt. rend. Acad. Sc. (1923) 177, 717.
18. Sellards and Bigelow, Journ. Med. Res. **42** (1920–21) 241.
    Sellards, Medicine **3** (1924) 99.
19. Kusama, Japan Med. World **5** (1925) 309.
20. Debre, Joanmon, and Papp, Ann. de méd. **20** (1926) 343.
21. Baron, Medizinische Klinik **23** (1927) 48.
22. Rosenau, Preventive Medicine and Hygiene. Appleton & Co., New York and London (1927).
23. Tunnicliff and Hoyne, Journ. Infect. Dis. **38** (1926) 48; Journ. Am. Med. Assoc. **87** (1926) 2139.

RUBELLA: RÖTHELN: GERMAN MEASLES

Definition.—German measles is a specific, infectious disease of a mild nature; it is characterized by a cutaneous rash which usually appears without prodromal symptoms. Its mortality, if any, is exceedingly low, and in the absence of any demonstrable causative agent it has been thought to be caused by a filterable virus.

History.—From our knowledge of the history of rubella it is believed that German measles probably existed in ancient times and was confused with scarlet fever and rubeola. Vaughan(1) states that—

> Capable students of the history of epidemiology claim that there is some evidence of the recognition of this disease from measles in the writings of Arabian physicians of the ninth and tenth centuries. We are also told that in the seventeenth century the learned epidemiologist of Sicily, Ingrassias, recognized that occasionally he had to deal with a disease resembling measles but to which an attack of measles gave no immunity. During the eighteenth and a large part of the nineteenth centuries there was much discussion as to the identity or the specificity of rubella and rubeola. These terms were used indiscriminately, and it is now quite evident that they were often transposed by certain authors.

Vaughan believes that the malignant epidemics attributed to rubella by German authors in the eighteenth century were undoubtedly epidemics of scarlet fever. The exact date when German measles was recognized as a separate and distinct disease is not known, although Vaughan states that—

> In 1815 Maton clearly pointed out the difference between scarlatina, rubella, and rubeola. Rubella was given a variety of names; in fact, nearly every clinician who wrote upon the exanthemata of infancy and childhood, for 100 years gave some new name to it. The term rubella was suggested by an English physician, Veale, in 1866 and soon found its way into the medical dictionaries. The name is especially appropriate, being the diminutive of rubeola and expressing at one and the same time the slight import of the disease and its relationship to measles; in other words, rubella means little or light measles.

Distribution.—German measles has long been known throughout Europe and the Americas. No doubt its distribution is limited solely to the distribution of its natural host, which is man.

The virus of German measles.—The cause of German measles is not known. Tunnicliff (see Measles) has found a streptococcus in measles which is different from the streptococci found in rubeola, but so little study has been made of this organism and its relation to German measles that only passing mention of it may be made at this time. German measles, being of such low mortality and of such a mild clinical nature, has received very little study from the standpoint of etiology. That rubella is a disease distinct from rubeola there can be no doubt. Measles gives no immunity to rubella, and in rubella Koplik spots are absent. In the absence of a definite etiologic agent and based upon the possible filterable nature of the virus of rubeola it is also thought that rubella may be due to a filterable virus.

Incubation period.—The period of incubation in rubella is usually from fourteen to twenty-one days but is subject to great variation.

Symptoms.—Usually there are no prodromal symptoms, although in some cases there may be mild constitutional symptoms before the rash appears. As a rule the first sign of the presence of this disease is the appearance of a rash. The rash is usually composed of very small maculopapules which are pale red in color, discrete, and the size of a small pea. The rash resembles rubeola in many respects and is subject to variation. In some cases the rash is hæmorrhagic and may have a "shotty" feel to the touch. The temperature usually does not exceed 101° F. and may fall abruptly as the rash disappears. In some cases the temperature may reach 103° F. and catarrhal symptoms with sore throat may be present. One of the most constant features of rubella is the enlargement of the cervical lymph glands. This swelling subsides slowly in most cases without suppuration, but in some cases it may persist for long periods of time. I have recently seen a case in which the cervical adenitis has persisted for nearly two years despite all the treatment to reduce it. Forcheimer(2) has described an enanthem of the mucous membrane of the throat in German measles consisting of minute, red points appearing upon the uvula and soft palate which he believes is characteristic of the disease. According to this author these "points" disappear within the first twenty-four hours of the disease.

Duke has attempted to differentiate between two forms of German measles, one of which closely resembles scarlet fever. This disease is known as "Duke's disease" or the "fourth disease." Opinion is not crystalized upon this matter.

In some cases there is no desquamation following the disappearance of the rash, while in others it may be fairly marked. The mortality in German measles is practically nil; complications are rare and when present are very mild in character.

Animals susceptible to rubella.—So far as is known man is the natural host of the infectious agent of this disease and is the only species affected. German measles has not been definitely produced in animals.

Immunity.—One attack of the disease confers a definite immunity against subsequent attacks. Rubeola and scarlet fever offer no protection against rubella and vice versa.

Pathology.—The anatomical changes in German measles are limited to the skin and the accompanying changes in the cervical lymph glands. Due to the mild nature of this disease there has been a great paucity of material for study, and in general it is believed that in uncomplicated cases of German measles the pathological changes are so mild that no importance should be attached to them.

Control measures.—According to Vaughan the evidence appears to favor the idea that German measles is transmitted chiefly through fomites. Authors are not agreed as to the degree of contagiousness of this disease, although it is generally admitted that it is contagious. Isolation is indicated, and in general the measures employed for measles should be instituted. Vaughan recommends the burning or disinfection of all articles with which the patient has been in contact.

BIBLIOGRAPHY

1. VAUGHAN, Epidemiology and Public Health. C. V. Mosby & Co., St. Louis (1922).
2. FORCHEIMER, Twentieth Century Practice of Medicine 14: 175.

OTHER REFERENCES

ROSENAU, Preventive Medicine and Hygiene. Appleton & Co., New York and London (1927).

HOLT, Diseases of Infancy and Childhood. Appleton and Co., N. Y.

EPIDEMIC PAROTITIS: MUMPS

Definition.—Mumps is a specific contagious disease characterized by an inflammation of the salivary glands. The parotid gland is usually involved and the submaxillary and sublingual glands may also be affected. In some cases the disease involves the testes in the male and the ovaries in the female. In some cases the first symptoms of the disease may be related to the testes. The disease is caused by a filterable virus.

History.—According to Vaughan(1) this disease has been known since the time of Hippocrates, though it was first recognized as a specific disease by Hamilton in Scotland in 1761. In discussing the epidemiology of this disease this author states that extensive epidemics of mumps occurred in Italy during the latter half of the eighteenth century. Mumps is an important disease in military organizations. Vaughan states that in 1918 there were 166,370 cases of mumps in the United States Army, and the loss of time caused by this disease has been estimated at 2,894,074 days. During the World War there was a total of 213,943 cases of mumps in the American Army. Considering the entire army at home and abroad for the year 1918

Vaughan states that 7,766 men were disabled by this disease for each day of the year. The total number of deaths for the year 1918 attributed to this disease was 151, the actual cause of death for the most part being a complication by pneumonia. In civilian life mumps usually occurs between the ages of 5 and 15 with most cases falling in the age group between 7 and 9 years. It is in this period of life, when the child starts to school, that he comes in contact with the virus from other cases. In armies the disease appears in the age group of 21 to 31 years chiefly. In the early history of mumps the disease was confused with secondary parotitis and practically nothing was known regarding the epidemiological features of the disease.

Distribution.—Mumps is distributed all over the world, and no place or country, it is thought, is ever continuously free from the disease.

The virus of mumps.—In the eighties of the nineteenth century various investigators reported bacterial forms in the blood and saliva of persons having mumps. Vaughan states that "in 1892 Laveran and Catrin found in 67 out of 95 cases of mumps micrococci resembling those already described. The testicular fluid obtained by puncture with a hypodermic needle gave positive results in 12 out of 16 cases. No inoculation experiments were made with these bacteria." Again, "in 1896 Mecray and Walsh found a diplococcus in the discharges from Steno's duct. . . . Granata (1908) for the first time, used filtered saliva in animal inoculations."

Granata used rabbits in his experiments, and his results are regarded as of doubtful significance. In 1913 Nicolle and Conseil,(2) working with filtered saliva from cases of mumps, found evidence of the experimental disease in the monkeys which they inoculated. These experiments were followed by the work of Wollstein(3) in 1918 which placed the filterable nature of this virus upon a sound basis. Wollstein inoculated the filtered mouth washings of children suffering from mumps directly into the parotid glands of cats. Following these inoculations the cats exhibited a small rise in temperature within forty-eight hours and slight local tenderness which Wollstein thought to be due to mechanical injury. In about seven days after the injection there was swelling and tenderness of the parotid and testicles. Wollstein states that "the swelling and pain in the parotid lasted from two to five days, but the testicular swelling rarely subsided in less than ten to fourteen days." There was a gradual increase in lecucocytes from the second day following the inoculation. Wollstein says:

> In the third week all the symptoms began to disappear, the leucocytes reaching the normal first, the tenderness disappearing at the same time, and the fever persisting for another week. While tenderness on palpation of the parotids was less marked than that of the testes, and the swelling never reached the stage of marked facial asymmetry, the cats manifested some degree of discomfort in the inoculated parotid. The appetite was only slightly affected and at no period of the experiment did the cats appear especially ill . . . the salivary filtrates from patients ill from one to three days produced these marked symptoms in the inoculated cats. On the sixth day of the disease the effect of the filtrate injection was much less marked and inoculation of material obtained from a patient nine days or longer after onset of the mumps attack was apparently without results.

Transmission of the experimental disease from cat to cat was successful with emulsions made of infected glands and by means of the saliva from inoculated cats.

Regarding the properties of this virus little is known. In view of the apparent ease with which cats can be infected with mumps virus it seems that much information on the nature of the virus may be obtained by further experiment.

Incubation period.—The incubation period of mumps may vary from three to twenty-five days, and on the average it ranges between fourteen and twenty-one days. Experimentally in cats, according to Wollstein, it is from six to seven days.

Symptoms.—Prodromal symptoms consisting of headache, anorexia, vomiting, back and leg pains, and fever may in some cases precede the local swellings. As a rule both parotids are involved and the jaws are painful before actual swelling is noted. Usually the swelling begins on one side, the other gland becoming involved one or two days later. All degrees of swelling may exist depending upon the severity of the case. The submaxillary and sublingual glands may also be involved. The salivary secretion is usually diminished, but in some cases there may be excessive salivation. The temperature ranges between 100 and 104° F. Venous congestion leading to hyperæmia of the brain with headache and delirium may result. Other complications such as orchitis, nephritis, deafness, pneumonia, meningitis, endocarditis, and suppuration may occur in some cases. However, these are rare. The blood picture in mumps is quite characteristic. It consists of a reduction in the polymorphonuclears and an actual and relative increase in the lymphocytes.

Animals susceptible to the mumps virus.—Man appears to be the natural host for this virus. Cats can be experimentally infected. The work on rabbits and monkeys is indefinite.

Immunity.—One attack of the disease confers a definite and in most cases a lifelong immunity to subsequent infection with this virus. The age incidence of mumps indicates that there is very little, if any, natural immunity to the disease.

Pathology.—The anatomical changes in this disease are found principally in the salivary glands. There has been a paucity of material for the study of pathological lesions in this disease, but it is quite generally known that the salivary glands exhibit hyperæmia and œdema, and the walls of the ducts are swollen and obstructed. Pyogenic germs may gain entrance and lead to abscess and suppuration, but this is rare. Other lesions depend upon various complications of the disease.

Control measures.—Isolation and disinfection are indicated as the most efficacious measures for the control of mumps.

BIBLIOGRAPHY

1. Vaughan, Epidemiology and Public Health. C. V. Mosby, St. Louis (1922).
2. Nicolle and Conseil, Comp. rend. Acad. Sc. 157 (1913) 340.
3 Wollstein, Journ. Exp. Med. 24 (1916) 265; 27 (1918) 337.

TRACHOMA: GRANULAR CONJUNCTIVITIS

Definition.—Trachoma is a specific, contagious disease, characterized by inflammation and hypertrophy of the conjunctiva; and by the formation of granules, with subsequent cicatricial changes. It is usually of long duration, occurs at all ages, is frequently complicated, and may lead to partial or total blindness. It occurs in three forms; namely, the papillary form, the granular form, and the mixed form.

History and distribution.—Trachoma has been known since ancient times. It has long been endemic in Egypt and is thought to have been carried to Europe by soldiers during the Napoleonic wars. It occurs in Arabia, Belgium, Holland, and Hungary. It is present in Italy and is found to be an important affection among the American Indians in sections of the United States. It is common among the Russians, Polish Jews, Hungarians, Italians, and Irish. It has not been common among the negroes. In the Philippines there is a form of follicular conjunctivitis which is endemic among school children in Pangasinan Province. This is probably not true trachoma, but extensive investigations into the true nature of this affection have not been made. Trachoma seems to have been introduced into the Netherlands about 1860 and is thought to have been carried to Amsterdam by Polish Jews. In a survey made in 1880 it was found that 45 per cent of 2,733 Jewish children were affected. By 1901 through periodic examination and control methods this percentage had fallen to less than 8 per cent. In China, Japan, Egypt, and Russia the disease remains a great public-health problem.

The virus of trachoma.—In 1907 Prowazek and Halberstaedter(1) described inclusion or trachoma bodies in the conjunctival epithelium of cases of trachoma. The exact nature of these bodies remains undetermined. These inclusions consist of coccoid and minute granular bodies. The coarser coccoid bodies stain bluish by the Giemsa method, while the granular forms stain reddish. Scrapings from the conjunctiva injected into the eye of the orang-utan produced conjunctivitis associated with the appearance of similar inclusion bodies. These bodies were regarded by these authors as the cause of trachoma. Later the same workers found similar inclusions in cases of uncomplicated blennorrhœa neonatorum, and their specificity was questioned by contemporary investigators. Herzog(2) suggested the theory that the gonococcus is transformed into small forms within the ephithelial cells and that the so-called trachoma bodies are in reality changed gonococci. Williams(3) regarded the inclusion bodies as degenerated forms of the Koch-Weeks bacillus. Prowazek regarded the trachoma bodies as protozoan in nature, while Noguchi(4) in 1913 claimed to have cultivated these bodies, although his attempts to induce trachoma in monkeys (*Macacus* and *Papio*) with his cultures failed. The exact relation of the so-called trachoma bodies to the true etiology of this disease, then, has remained undertermined. Because of the uniform presence of these inclusions in typical uncomplicated cases of the disease and the occurrence of similar bodies in various other diseases which are thought to be caused by filterable viruses, and in the absence of any other definite etiological agent, the possibility of a filterable virus etiology has been suggested for trachoma. According to Rosenau,(5) "experimental evidence permits no more than the suspicion that the virus may be filterable under some circumstances." However, fairly convincing evidence has been presented upon this point in the experiments of Bertarelli and Cechetto(6) and of Nicolle, Cuénod, and Blaizot.(7)

Heymann(8) after finding inclusions in cases of gonorrhœal blennorrhœa neonatorum suggested that the so-called inclusion bodies were in reality reaction products of the gonococcal virus. Simon(9) states:

> A thorough study of this question then led to the interesting discovery of the existence of an inclusion blennorrhea as a malady *sui generis*, which primarily affects the genitalia of both male and female and secondarily the eyes of the new born. This type of blennorrhea it is now known may be associated with a gonococcal infection, as well as with other bacterial infections (pneumococci, diphtheria bacilli), but when this occurs the processes are independent of each other.
>
> The discovery of the occurrence of inclusions in connection with blennorrhea of this type naturally threw doubt upon the correctness of Prowazek's view, that the constituent granules making up the inclusions found in trachoma actually represented the trachomatous virus. Various suggestions have accordingly been made to account for their appearance in trachoma, on the one hand, and in inclusion blennorrhea, on the other.

Linder(10) inoculated two baboons with pure inclusion blennorrhœal material and obtained a clinical and histological picture which he states cannot be distinguished from trachoma. Wolfrum(11) inoculated similar material into a human being, and there followed the typical picture of trachoma. Simon suggests the theory that the inclusion bodies may not be part of the picture of trachoma but are found in cases of this disease only when both blennorrhœa and trachoma are present in the same subject.

In 1927 Noguchi(12) produced an experimental trachomalike condition in monkeys with material obtained from cases of trachoma from Indians at the government school for Indians at Albuquerque, New Mexico. From this material he cultivated upon special media a Gram-negative bacillus which when injected into monkeys produced a granular conjunctivitis that "had an appearance strikingly like that of the human trachomatous conjunctiva in the early stages of the disease." This organism was associated with four of the five cases studied, and the conjunctival disease produced is said by Noguchi to be transmissible in series while the identical microörganism is obtained regularly even in the second and the third passage in monkeys. None of the other organisms isolated by Noguchi from his material was capable of inducing follicular lesions in monkeys. Material taken direct from patients and injected into monkeys did not produce lesions within four months.

This work of Noguchi's represents an important contribution to the knowledge of trachoma as it exists in the American Indian but must, of course, await confirmation in other localities.

Incubation period of trachoma.—Trachoma is a chronic disease. Its incubation period is not definitely known. The recognition of the disease in its early stages is very difficult, and accurate diagnosis usually depends upon changes that appear later in the course of the disease. In the experimental form of the disease, produced by Noguchi in monkeys, the first changes were noted two to four weeks after inoculation with the cultures obtained from trachomatous material.

Symptoms.—There are three forms of granular conjunctivitis; namely, papillary, granular, and mixed forms. Regardless of the form, there are certain subjective symptoms which may be present; such as, photophobia, lachrymation, itching and burning sensations, feeling of a foreign body, pain, and visual disturbance. In some cases there may be no subjective symptoms. The objective symptoms consist of swelling of the lids, narrowing of the palpebral aperture, and dropping of the upper lid. There may be a mucopurulent discharge and the conjunctiva of the tarsus and fornix is reddened, thickened, and uneven, on account of the hypertrophy and the occurrence of granules. Trachoma progresses slowly up to a certain point, then is followed by the cicatricial stage. The papillæ and granules disappear, but the conjunctiva does not return to normal and various degrees of scarring remain. The entire surface of the conjunctiva may be replaced by a cicatricial membrane.

In some cases the condition is acute and is accompanied by marked inflammatory symptoms. Gonococcal infection may be associated with true trachoma; and the diagnosis may be difficult, especially early in the course of the disease. In other cases the symptoms may be so mild and the disease so insidious that it may exist for months without recognition. In fairly severe cases there may be intermissions and exacerbations, and relapses are quite frequent. The disease is frequently complicated by pannus and corneal ulceration. The commonest sequelæ are trichiasis and entropion, ectropion, symblepharon, corneal opacities, staphyloma of the cornea, and xerosis.

Animals susceptible to the virus of trachoma.—Man is the natural host for the virus of trachoma. From experimental sources it may be said that the evidence points to the possibility of infecting monkeys.

Immunity.—That there is a definite racial predisposition to infection with trachoma is borne out by the studies on the epidemiology of this disease. It may be assumed by the same token that there exists some degree of racial immunity and, for that matter, an individual natural immunity. There is, however, no experimental evidence bearing upon this point. Immunity has not been produced experimentally.

Pathology.—In the papillary form a large number of small elevations appear upon the conjunctiva giving a velvety appearance, and if the papillæ are larger, a granular appearance. This form occurs only upon the upper lid. The papillæ represent the hypertrophied conjunctiva thrown into folds and covered by increased epithelium. Within there is a cellular infiltration. In the granular form there are grayish, rounded, translucent bodies or granules which are seen through the conjunctiva. These bodies may be small and round, large and warty, or flattened and succulent. They are principally in the fornix. They may also be found upon the semilunar folds and the bulbar conjunctiva. These granules represent collections of lymph corpuscles in a connective-tissue reticulum, resembling Peyer's patches in the intestines. According to Noguchi the histologic changes present in trachoma as it exists in the American Indian are as follows:

> The essential features of the lesions in the human disease are (1) diffuse infiltration of lymphocytes mingled with plasma cells, extending along the entire length of the subepithelial or adenoid layer; (2) the presence of fairly well defined follicles, consisting of layers of lymphocytes, enclosing a mass of large round or polyhedral epithelioid cells with paler staining cytoplasm and nuclei; (3) ill defined foci of mingled lymphocytes and large epithelioid cells; (4) the presence of Lebeer cells within the follicle and elsewhere; (5) the presence of fine connective tissue fibrils surrounding or penetrating the infiltrated or follicular areas, and (6) the proliferation of the conjunctival epithelium, which in some places shows several layers of flattened cells, and in others is thinned out to a single layer or even ruptured by protruding follicles. In older lesions the infiltration and follicles have given place to increased numbers of connective tissue fibers, which bind the epidermized conjunctival epithelium to the often deformed tarsus. A few polymorphonuclear leukocytes may be found in the tumid epithelial layer, but their presence is not usual.

In the experimental disease produced in monkeys Noguchi found similar changes.

Control measures for trachoma.—Trachoma has always been associated with poverty and squalor. Unhygienic conditions predispose to the disease. Early diagnosis of the condition is important in order to prevent serious sequelæ. The secretion from the eyes of trachoma patients is regarded as contagious, and the disease may be transmitted by infected handkerchiefs, towels, washbasins, etc. Isolation of trachoma cases has been advocated, especially during epidemics. In general, early diagnosis of the disease coupled with intelligent care and the institution of strict sanitary measures are indicated.

BIBLIOGRAPHY

1. PROWAZEK and HALBERSTAEDTER, Deutsch. med. Wchnschr. **33** (1907) 1285; Arb. a. d. k. Gandhtsamte **26**: 44; Berl. klin. Wchnschr. **46** (1909) 1110.
2. HERZOG, Arch. f. Ophth. **74** (1910) 520.
3. WILLIAMS, Arch. f. Ophth. **42** (1913) 506.
4. NOGUCHI, Journ. Exp. Med. **18** (1913) 572–578.
5. ROSENAU, Preventive Medicine and Hygiene. Appleton & Co., New York and London (1927).
6. BERTARELLI and CECHETTO, Centbl. f. Bakt., O. (1913) 70.
7. NICOLLE, CUENOD, and BLAIZOT, Compt. rend. Acad. Sc. (1912) 241; (1913) 1177.
8. HEYMANN, Deutsch. med. Wchnschr. **35** (109) 1692.
9. SIMON, Physiological Reviews **3** No. 4 (1923) 483–508.
10. LINDER, Wien. klin. Wchnschr. (1909) 1555, 1659; v. Graefe's Arch. f. Ophthal. (1913) 84.
11. WOLFRUM, 36te Versammlung d. ophthal. Ges. Heidelberg (1910) 207.
12. NOUGUCHI, Journ. Am. Med. Assoc. **89** (1927) 739–742.

INCLUSION BLENNORRHŒA: INCLUSION CONJUNCTIVITIS

Following the work of Heymann in 1909 (see Trachoma) who found in several cases of gonorrhœal blennorrhœa neonatorum inclusions similar to those that had been described in trachoma by Prowazek, it appeared that there existed a separate and distinct type of blennorrhœa not associated with gonorrhœa or trachoma. This type of blennorrhœa primarily affects the genitalia of both male and female and secondarily the eyes of the new born. While this condition may exist along with a gonorrhœal or other bacterial infection, it is now recognized that the processes are independent of each other. The cause of this form of blennorrhœa is unknown. Inclusion bodies are found within the lesions which suggests a filterable virus origin for the disease.

Inflammations of the conjunctiva are of several varieties and generally are divided into the following types: Catarrhal (acute, chronic, and follicular); purulent (ophthalmia neonatorum, and gonorrhœal); membranous (nondiphtheritic or croupous, and diphtheritic); granular (trachoma); and phlyctenular. It is well recognized by ophthalmologists that there are cases of ophthalmia neonatorum which are not caused by gonococcal infection, and these are believed to be due to infection with simple catarrhal (nongonorrhœal) secretion. In 1913 Cohen(1) reported on the clinical course of conjunctival affections associated with so-called trachoma bodies which was a further study of the cases described in an earlier paper by Noguchi and Cohen(2) published in 1911. The original cases studied by these authors included nine cases of trachoma represented by four stages of the disease, six cases of blennorrhœa neonatorum nongonorrhoica, and six cases of blennorrhœa gonorrhoica in young girls. As a result of these cases there were a number of other cases infected. There were nineteen new cases of trachoma, two new cases of blennorrhœa neonatorum (nongonorrhoica), and twenty cases of blennorrhœa gonorrhoica in young girls. Inclusion bodies were found in the six cases of blennorrhœa neonatorum nongonorrhoica varying from four days to two weeks after birth. Cohen states that—

> The clinical course of these cases resembles that of mild cases of blennorrhœa gonorrhoica, which in its earliest stage is characterized by a diffuse conjunctival congestion with a mucoid secretion from the conjunctiva. The condition remains for about one week, when the conjunctiva assumes a fine papillary appearance, and a few small follicles are seen on the upper fold as well as on the lower. This appearance lasts about two months, when the process regresses simultaneously with the gradual disappearance of the bodies and is followed by a permanent return of the conjunctiva to normal in from three to four months.

In one of Cohen's cases, so-called trachoma bodies were found in an affected eye from the mother and these were demonstrated at intervals for three months. In his study of thirty cases of blennorrhœa gonorrhoica in young girls at Randall's Island Hospital, Cohen was able to demonstrate gonococci and so-called trachoma bodies in practically every case. These bodies persisted even after the gonococci could no longer be found. Likewise in his true trachoma cases Cohen found inclusion bodies.

The interesting feature of this study is the fact that inclusion bodies are found in cases of blennorrhœa which are of neither trachomatous nor gonorrhœal origin. These cases are not trachoma since they bear no clinical resemblance to trachoma and because there is spontaneous cure without sequelæ. In Cohen's opinion "where bodies were found in conjunction with gonococci, and in some cases of typical trachoma, these conditions are to be interpreted as the result of the disease caused by these bodies becoming engrafted on the original affections." Cohen believes that the term "trachoma bodies" is a misnomer and should be discarded.

While there is nothing known regarding the etiology of this condition there has been a tendency to classify inclusion blennorrhœa with the filterable virus diseases. At present there is no experimental evidence that it is caused by a filterable virus.

BIBLIOGRAPHY

1. COHEN, Arch. of Ophthal. 42 (1913) 29–33.
2. NOGUCHI and COHEN, Arch. of Ophthal. 40: 1.
See also Trachoma.

VERRUCÆ: WARTS

VERRUE (FRENCH); WARZE (GERMAN)

Definition.—Verrucæ, or warts, represent an epidemic, papillary new growth of which there are three recognized types; namely, verruca vulgaris, verruca plana juvenilis, and verruca senilis.

History.—Warts have been recognized since olden times, and legends and superstitions have been connected with their appearance for perhaps centuries. Even to this day there are certain peoples who are prone to regard these lesions with various superstitions. The infectious nature of these growths was demonstrated in 1891 when Payne(1) developed warts under his thumb nail following the removal of warts from one of his patients. Lanz(2) reported similar results in 1898. In 1889 Kuhneman(3) cultivated a bacillus from warts and claimed to have reproduced typical lesions in laboratory animals. Variot(4) four years later produced warts in one of his assistants following the inoculation of blood from small warts. During the next year Jadassohn(5) made seventy-four inoculations with wart material from which he obtained thirty-one positive results and demonstrated that the lesions he produced were typical verrucæ according to their histologic picture. In these experiments this author demonstrated that the incubation period for warts ranges from seven weeks to three months. In 1919 Wile and Kingery(6) reported their brilliant experiments which proved conclusively that warts are due to a filterable virus.

The virus of verruca.—Wile and Kingery began their experiments on the theory that the infectious agent of warts is a filterable virus. In their first paper they point out that certain microörganisms are known to give rise to disorders of keratinization. Examples of this fact, such as the rôle of the gonococcus in the production of blennorrhagic keratoses, the tubercle bacillus in verruca necrogenica, and the gonococcus in the production of condyloma acuminatum, are mentioned.

Their experiments consisted of the removal of clinical warts and grinding this tissue in a small amount of saline after which the saline emulsion was filtered through the finest Berkefeld filter. In order to obtain the maximum amount of material for their experiments the filter candle was almost entirely covered with melted paraffin leaving only the top end exposed as a filtering surface. After testing their filtrate for sterility small amounts of filtrate were inoculated intradermally into human subjects. Part of their material was preserved in glycerin to be tested later. In the course of about four weeks small wartlike growths appeared in one subject, while a second showed lesions one week later, and a third about three weeks later. In only one case did a wart reach the size of a large pea. This occurred in about eight weeks. In some cases there was a tendency to spontaneous resolution; however, in most cases the lesions persisted for at least seven months. The histologic studies made upon these new growths were typical of true warts. A control which received a filtrate prepared with normal epithelium remained negative. In later experiments the preserved material in glycerin was tested in a similar manner, but results with this material were negative after nearly six months. These authors concluded that "the sterile filtrate of wart material injected intracutaneously is capable of producing localized hyperkeratoses which are clinically and pathologically identical with verruca vulgaris."

In 1921 this work was further extended by Kingery(7) when he demonstrated that lesions could be produced in the second generation from the initial lesions described above. In these experiments the incubation period was found to be nearly six months. There are no data on the properties of the virus of verruca.

Symptoms.—The lesions of warts are unaccompanied by subjective symptoms. When they first begin to appear they are small, flat, shiny areas which increase slowly in size. Later the growth may present a distinct papillary surface. At first it is the color of the skin, then gradually becomes grayish and even grayish black. As a rule there is no pain or itching except when inflammation is present.

Immunity.—There are no data on immunity in this form of new growth. While no experimental evidence is available on the subject, it is generally assumed that there is a natural immunity to the virus which varies greatly in degree. This is indicated by the variable period of incubation and the fact that most individuals rarely become infected with this virus.

Pathology.—Histologically warts are characterized by a typical localized acanthosis. The growths begin as an early hyperkeratosis which gradually becomes more marked. In the late stages there is a proliferation of the papillary tufts which later thicken and dip down. In general all the layers of the epidermis are more or less increased in thickness. The granular layer in increased, the rete cells are enlarged, and the intercellular spaces are widened. In some cases there is moderate inflammation, and round-cell exudate is found in the neighborhood of the vessels. All of these changes vary according to the type of growth.

Control measures.—None are indicated. These growths are benign. Rarely do they become epitheliomatous.

BIBLIOGRAPHY

1. PAYNE, Brit. Journ. Dermatol. 3 (1891) 185.
2. LANZ, Cor.-Bl. f. Schweiz. Aerzte. (1898) 264.
3. KUHNEMAN, Monatsh. f. prakt. Dermatol. 9 (1889) 17.
4. VARIOT, Journ. de clin. et de thérap. inf. 94 (1893) 892.
5. JADASSOHN, Arch. f. Dermatol., 5th Congress (1896).
6. WILE and KINGERY, Journ. Am. Med. Assoc. 73 (1919) 970.
7. KINGERY, Journ. Am. Med. Assoc. 76 (1921) 440.

OTHER REFERENCES

KYRLE, "Histo-biologie der Haut." Berlin, Springer (1925) fig. 46.
LIPSCHÜTZ, Wien. klin. Woch. 37 (1924) 286.
SANGIORGI, Cbl. f. Bakt. 76 (1915) 257.

MOLLUSCUM CONTAGIOSUM: EPITHELIOMA

MOLLUSCUM SEBACEUM; MOLLUSCUM EPITHELIALE; ACNE VARIOLIFORMIS

Definition.—Molluscum contagiosum is regarded as a contagious epithelial neoplasm, or new growth, which is characterized by small tumors the size of pinheads or peas, usually the color of normal skin but at times pinkish or bright red, with a small depressed central opening. These new growths are believed to be caused by a filterable virus.

History.—The term "molluscum," or "molluscis," is thought to have been first employed by Ludwig(1) in 1739 as a synonym for "mollis" to indicate certain soft tumors, while others believe that the word was used because of the resemblance of certain cutaneous tumors to knots on the bark of the maple. The first clinical description of this disease was given by Bateman(2) in

1817, while Patterson(3) in 1841 studied the secretion from molluscum tumors and called attention to the so-called molluscum corpuscles or bodies. This author believed that these bodies represented nuclei. In 1844 these growths were regarded by Engel(4) as enlarged sebaceous glands, a view which was concurred in by Rokitansky(5) in 1856. Virchow(6) in 1865 regarded molluscum tumors as a lobulated glandular epithelioma. He believed that the molluscum bodies arose from the hair follicles and likened their appearance to swollen starch bodies and fatlike globules, although he thought they were probably the result of a degenerative process involving the epithelium. In later years Bizzozero and Manfredi(7) contended that these peculiar bodies originated from the protoplasm of the cell; Retzius(8) affirmed that they were sui generis, that their size precluded the idea that they could be spores or parasites; Boeck(9) stated that the bodies arose from peculiar epidermal cells, a metamorphosis of the rete cells, and that according to his chemical tests these cells contained no fat and were not amyloid; Lukumsky(10) suggested that the bodies came from cells which had invaded the epidermis. In 1878 Vidal(11) advanced the idea that the molluscum bodies were the product of colloid degeneration.

Angelucci(12) in 1881 described a bacterium, *Bacterium lepogenum,* as the cause of molluscum contagiosum, while Neisser(13) the following year claimed that the specific cause of the disease was a gregarine. In 1886 this author again stated his belief in the parasitic origin of the disease and stated that the molluscum bodies were in reality coccidia and related to the Sporozoa. Graham(14) in 1892 described a micrococcus as the cause of the disease, while the following year Neisser again confirmed his coccidial theory of the origin of the tumors. In 1902 White and Robey(15) recapitulated the trend of thought on the nature and cause of this disease. They pointed out that there are those who believe in the sebaceous origin of the tumors and also those who contend that the tumors originate in the rete. Further, that some authors believed in the contagiousness of the disease, while others were equally certain that it was noncontagious; that one school of thought considered the molluscum bodies as evidence of epithelial degeneration, while others considered these peculiar bodies as parasites. These authors isolated a staphylococcus from molluscum tumors but did not consider it of any etiological significance. They concluded by stating that until that time they considered that no one had isolated any parasitic body from the growth and that in their opinion the changes produced did not represent a colloid of hyaline degeneration but rather a metamorphosis of rete cells into keratin.

In 1909 Knowles(16) found what was apparently *Micrococcus salivarius* in a few cases of the disease but did not claim any etiological rôle for this organism. It was not until 1919 (nearly one hundred years after the disease was first described) that the epical work of Wile and Kingery(17) on the etiology of this disease appeared. According to these authors the disease is due to a filterable virus. Juliusberg(18) had suggested this possibility in 1905, but the evidence he presented did not substantiate his claims. In 1923 Clarke(19) described a parasite grown from molluscum lesions which he named *Plassomyxa contagiosa* and which he believes to be the cause of this disease.

The virus of molluscum contagiosum.—Wile and Kingery not only demonstrated that the virus of molluscum contagiosum is filterable but also succeeded in producing experimentally in human beings typical tumors with the sterile filtrate of typical lesions. These authors further showed that the incubation period of the disease varies with the individual's predisposition or susceptibility; in one case it was found to be fourteen days while in another it was twenty-five days, and the microscopical diagnosis was made at the fifty-fifth day. These authors believe that the molluscum body develops late in the stage of evolution of the tumor and further that it represents a degenerative stage in this evolution.

Symptoms.—The tumors of molluscum contagiosum are quite solid and contain a cheesy material which can be pressed out of the growth through the central opening. In some cases the tumor mass extrudes this material spontaneously. Usually the lesions of molluscum are found on the face, around the eyelids, or in the neighborhood of the genitalia, or elsewhere on the body. They rarely occur on the soles of the feet or the palms of the hands. Usually there are only few lesions, a few or a dozen or more, though in some cases they may be very numerous. They are discrete but in some cases several tumors may coalesce. They may become inflamed, or suppurate. In some cases there may be severe itching but this is not common. The lesions may persist for several months or even years in rare cases. While the lesions are usually limited to the skin there have been cases reported in which lesions have occurred upon the tongue and other mucous membranes. The infection is more frequent in children than in adults and may be transmitted by infected towels or gymnasium mats, etc. It is known to be transmitted directly from person to person in some instances.

Animals susceptible to the virus of molluscum contagiosum.—The disease is primarily a disease of man but a similar disease has been described in animals, especially in domestic fowls such as the pigeon. In a few cases the disease is known to have been transmitted from animals to human beings. Dogs and pigeons are both said to have transmitted the disease to man.

Immunity.—Little is known regarding immunity in this disease. Cases are known to have developed lesions of molluscum several times. That there is a natural immunity to the disease is indicated in the work of Wile and Kingery, who point out that there is a difference in the predisposition or susceptibility of the individual. From the experimental standpoint no conclusive data are available.

Pathology.—The tumors of molluscum contagiosum are essentially epithelial neoplasms. They are surrounded by a thin fibrous capsule and contain lobules of epithelial cells which are separated by thin septa and open upon the surface of the skin through a depressed central opening. It has become a generally accepted view that the tumors arise from the rete since the cells on the periphery of the lobules are of the type found in the basal layer of the rete. The central oval cells contain the so-called molluscum bodies which are regarded by Wile and Kingery as a degenerative stage in the evolution of the tumor. Lipschütz has called a minute organism found in the epithelial cells *Strongyloplasma hominis* which conforms to a general classification of peculiar bodies described by this author (see Introduction). According to modern textbook description three kinds of degenerated cells may be observed in these lesions. First there are large round bodies which contain an eccentrically placed nucleus; then there are oval cells surrounded by normal epithelium which contain a nucleus lying at one pole of the cell; and finally completely degenerated cells which are oval, structureless bodies. The exact nature of the degenerative process is still unknown.

Prevention.—The disease is comparatively trivial and is of no great importance either to the individual or to the community. While personal hygiene, discouragement of the use of the common towel, etc., are indicated, the chief effort should be directed to the proper treatment of the disease in order to eliminate carriers of the infection.

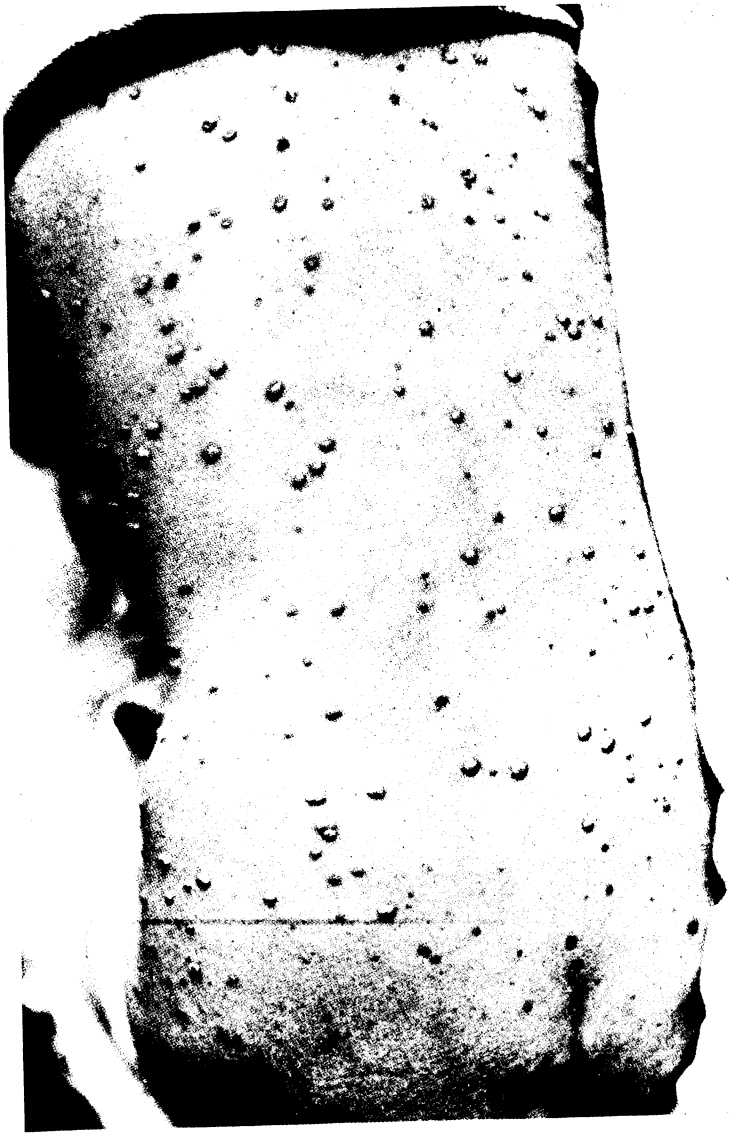

PLATE 5. VARICELLA.

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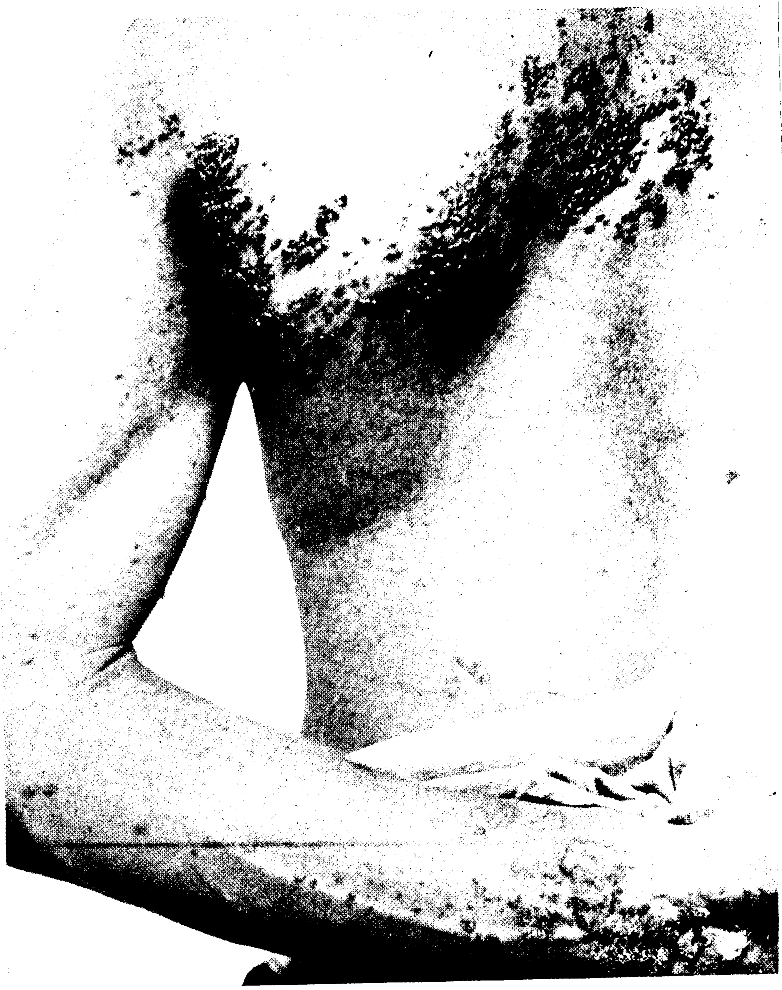

PLATE 6. HERPES ZOSTER ARSENICALIS-LICHEN PLANUS ON FLEXOR SURFACE OF WRIST.

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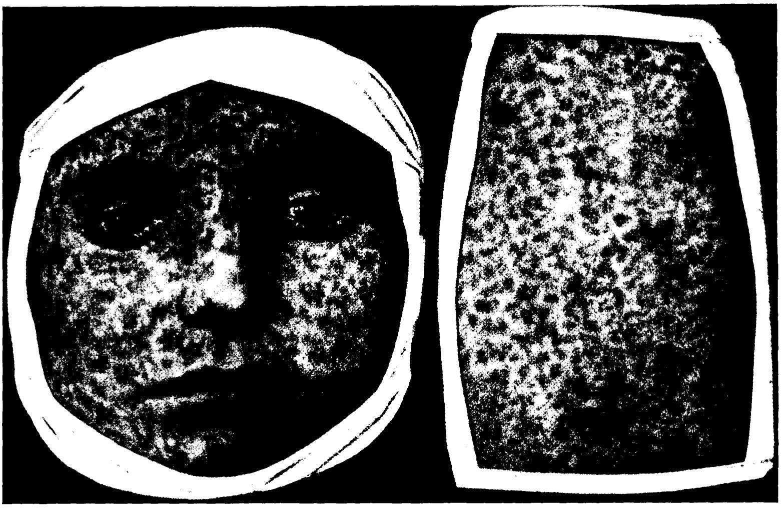

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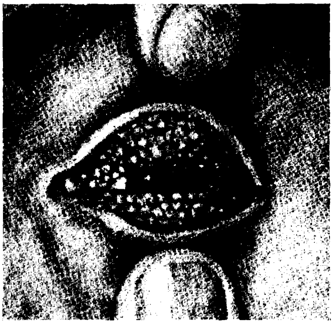

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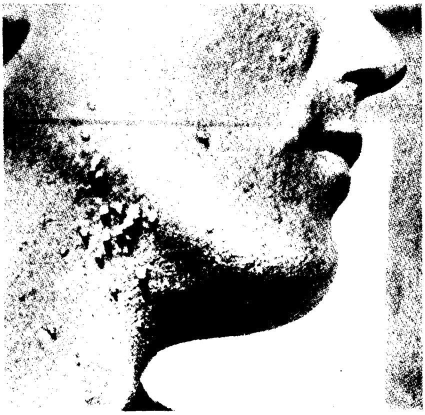

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Fig. 1. Measles. 2. Granulated conjunctivæ in trachoma. 3. Verrucæ digitatæ.

PLATE 7.

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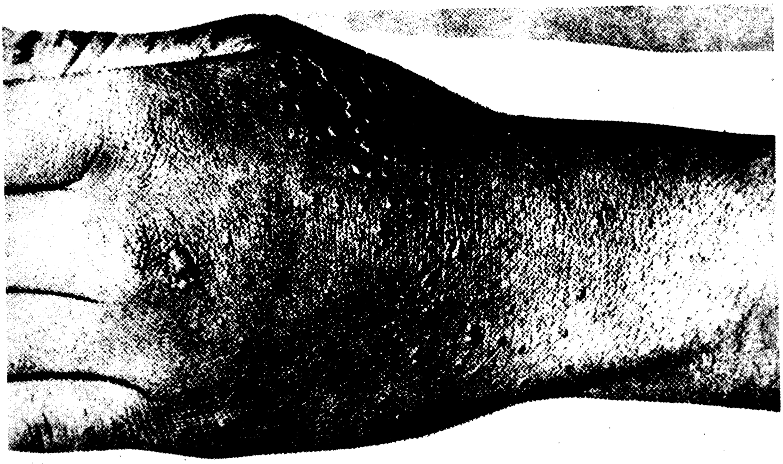

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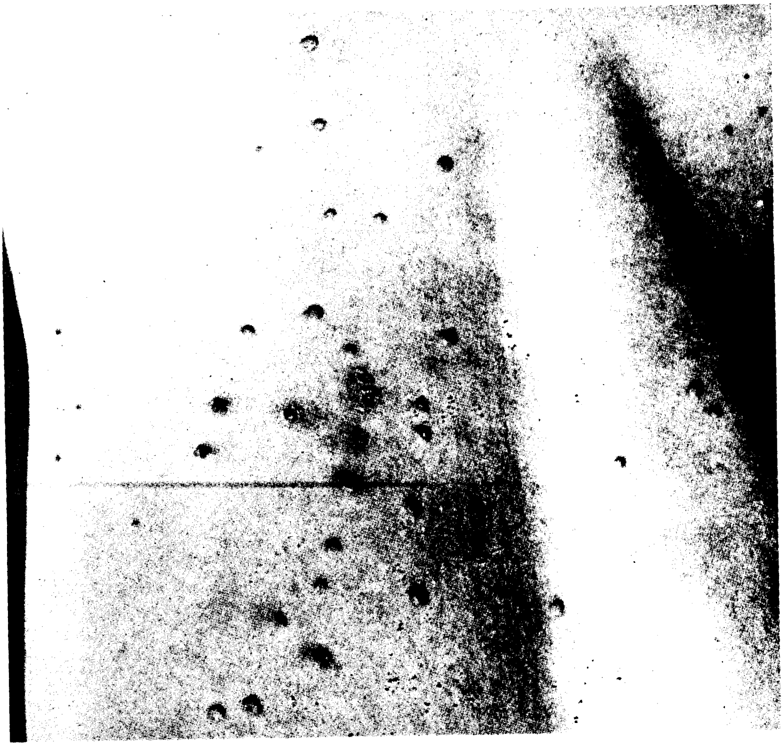

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Fig. 1. Verruca plana juvenilis. 2. Molluscum contagiosum.

PLATE 8.

UNIV. OF MICH.

BIBLIOGRAPHY

1. LUDWIG, Quoted from Knowles in Journ. Am. Med. Assoc. **53** (1909) 671.
2. BATEMAN, Delineations of Cutaneous Diseases (1817).
3. PATTERSON, Edinburgh Med. and Surg. Journ. (1841) 280.
4. ENGEL, Zeit. d. k. k. Gesellschaft der Aerzte in Wien (1844).
5. ROKITANSKY, Pathologische Anatomie (1856) 79.
6. VIRCHOW, Berl. klin. Woch. (1865) 34; Virchow's Archiv 144.
7. BIZZOZERO and MANFREDI, Arch. f. Dermat. (1871) 599; (1876) 6510.
8. RETZIUS, Deutsche Klinik. No. 50 (1872).
9. BOECK, Viertaljahresschrift f. Dermatol. und Syph. (1875) 23.
10. LUKOMSKY, Virchow's Arch. (1875) 145.
11. VIDAL, Le Progrés Medical (1878) 478.
12. ANGELUCCI, International Medical Congress (1881).
13. NEISSER, Monats. f. prakt. Dermat. (1882); Vierteljahresschrift f. Dermat. u. Syph. (1888) 553; Verhandlungen der deutschen dermatologischen Gesellschaft, IV. Congress (1893) 589.
14. GRAHAM, Journ. Cutan. and Gen. Urin. Dis. (1892) 89.
15. WHITE and ROBEY, Journ. Med. Res. n. s. 2 (1902) 255–277 (lit.).
16. KNOWLES, Journ. Am. Med. Assoc. **53** (1909) 671.
17. WILE and KINGERY, Journ. Cut. Dis., Chicago **37** (1919) 431.
    KINGERY, Arch. Dermatol. and Syph. 2 (1920) 144.
18. JULIUSBERG, Deutsch. Med. **21** (1905) 1598.
19. CLARKE, Brit. Journ. Derm. and Syph. **35** (1923) 24.

ILLUSTRATIONS

PLATE 5

Varicella. (After Hartzell.)

PLATE 6

Herpes zoster arsenicalis-lichen planus. Patient had a lichen planus shown on the flexor surface of the wrist, for which he had been taking Fowler's solution. (After Hartzell.)

PLATE 7

FIG. 1. Measles. (From Hartzell; after Pfaundler and Schlossman.)
2. Granulated conjunctivæ in trachoma. (After May.)
3. Verrucæ digitatæ. (From Hartzell.)

PLATE 8

FIG. 1. Verruca plana juvenilis. (After Ormsby.)
2. Molluscum contagiosum. (After Ormsby.)

CHAPTER IV

FILTERABLE VIRUS DISEASES OF MAN AND ANIMALS
(CONTINUED)

RABIES: HYDROPHOBIA

CANINE MADNESS; WUTKRANKHEIT, TOLLWUT (GERMAN); LA RAGE (FRENCH); RABBIA (ITALIAN)

Definition.—Rabies is an acute, rapidly fatal infection of the central nervous system. It is highly specific and a disease primarily of animals. It is transmitted directly from one animal to another, usually through a wound produced by biting. It is most prevalent among Carnivora but is infectious for nearly all Mammalia. Rabies is communicated to man from lower animals by means of infectious saliva introduced into the tissues through a biting wound or through small abrasions in the skin of the host. The disease in man is most commonly transmitted by dogs.

History.—Although the actual virus of rabies has not been demonstrated in the saliva of infected dogs, it was demonstrated by Zinke as early as 1804 that the saliva of rabid dogs is infectious. Seventy-five years elapsed after this discovery before Galtier(1) was able to show that the disease could be transmitted to rabbits through artificial inoculation (1879). In 1881, Pasteur(2) with Chamberland and Roux found that the virus has a special affinity for the central nervous system. The work that followed this discovery by Pasteur and his collaborators from 1881 to 1888 will remain forever a classical achievement in the science of modern medicine. During these years the method of immunization now in general use through the world was perfected by Pasteur, Roux, Chamberland, and Thuillier.(3) The story of this magnificent contribution to medical science is beautifully portrayed in mosaic in the tomb of Pasteur at the Institute Pasteur which stands as a monument to his brilliant work. It was not until 1903 that Renlinger(4) demonstrated the filterability of the rabies virus. This was confirmed in 1904 by Berterelli and Volpino(5) and later in 1913 in the

United States by Poor and Steinhardt.(6) Negri,(7) an Italian of Pavia, Italy, described in 1903 bodies found in the cells of the central nervous system. These bodies, which now bear his name, are found especially in the large ganglion cells of the hippocampus major and in the Purkinje cells. Negri's work has been amply confirmed by Volpino,(8) Da Mato,(9) Bertelli,(10) Bocs,(11) Poor,(12) and other investigators. In 1913 Noguchi reported the cultivation of Negri bodies, but his work has not been confirmed.

Distribution of the disease.—Rabies exists practically all over the world. It is commonest in France, Belgium, Russia, certain parts of the United States, and in the Philippine Islands. It is said never to have been present in Australia, and for more than fifty years has not been known in Denmark, Sweden, and Norway. Rabies was eradicated from England until the World War and then reappeared. It is said to have been reintroduced by dogs carried in aëroplanes. One of the largest rabies clinics known to the author is in the Philippine Islands, where there is a daily clinic at the Bureau of Science of about sixty patients reporting for immunization.

Incubation period.—There is no disease in which the incubation period varies more markedly. The usual period of incubation is from two to eight weeks, although cases are on record in which symptoms did not develop until two years had elapsed. Various explanations have been advanced to explain the latency of the virus in the tissues. The most plausable theory perhaps is the time required for the virus to travel up the axis cylinder of the nerve trunk to the central nervous system. Favorable conditions may not exist for the multiplication of the virus at the time of infection and the virus remains dormant though alive, awaiting optimum environment. Though purely theoretical this concept has its adherents. The incubation period in animals varies also, with the amount and the virulence of the virus, with the extent and type of the wound, and particularly with the location of the wound in relation to its nerve supply. The average incubation period for various animals is usually given as follows: Man, thirteen to sixty days; dogs, ten to forty days; cows, twenty-eight to fifty-six days; goats and sheep, twenty to twenty-eight days; pigs, ten to twenty-one days. For birds the incubation period varies from fourteen to forty days.

Symtoms of the disease.—Generally speaking, in dogs the first symptoms of the disease appear two to eight weeks after the wound. During the first stage of the disease (stadium prodromorum or melancholicum) there may be so little manifestation of symptoms that the condition is entirely unsuspected. The infected animal may be somewhat irritable and depressed, capricious, or may avoid all noise and activity and hide away in some dark place. On the other hand the animal may appear mildly excited, moving from one place to another, scratch with the fore feet, and without cause bark and bite at the air. Strange persons provoke growling and snapping, and slight stimuli often cause the animal to become startled or jump up frightened. One may observe during these paroxysms dilation of the pupils and slight respiratory disturbances. About this time the animal loses his appetite and later refuses all food. Difficulty in swallowing is quite characteristic, and although the animal seeks water he is able to swallow very little of it. By this time salivation becomes manifest and increases. Sexual desire is increased, and licking of the genitals is quite common. Within one to three days after onset of symptoms the animal passes into the excitable stage (stadium excitationis or acmes). The animal wants freedom, to be away from his usual surroundings. He will lick the ground, chew any object, and develop a violent rage. Once loose the animal runs aimlessly, often covering long distances and attacking people and animals along the way. The eyes are bloodshot. When fighting with other animals the infected dog remains quiet though the normal animal howls and barks. Infected dogs when caged remain quiet but develop a violent rage when teased. Frequently their teeth are broken in grabbing the bars of the cage and attacking objects with which they are teased. Such animals have been known to bite a red-hot iron or burning coal. Stages of exhaustion follow such attacks, and the animal falls down in unconsciousness for indefinite periods. About this time the early symptoms of paralysis appear. The larynx may first become paralyzed as evidenced by the peculiar hoarse bark. Swallowing becomes very difficult as the inflammation increases, and later there is degeneration of the eleventh and the twelfth pair of nerves. The animal refuses all food and drink and the sight of water may provoke an attack, hence the term hydrophobia. Circus movements have been noted in this stage. The paralysis gradually progresses and involves other parts of

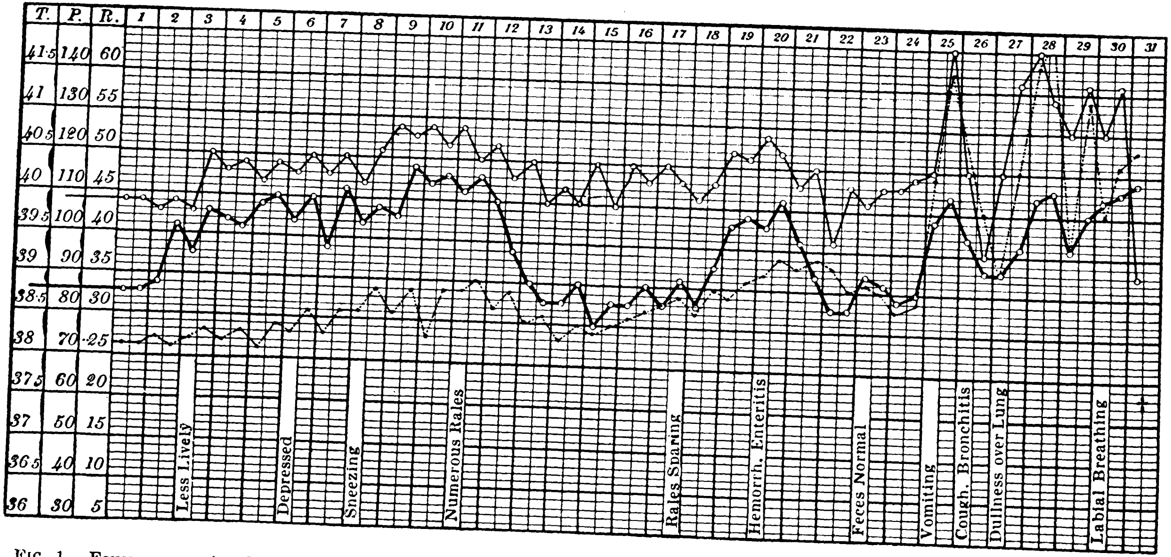

FIG. 1. Fever curve in dog distemper. Catarrh of the air passages, later gastroenteritis, toward the termination catarrhal pneumonia. (After Hutyra and Marek.)

the body. The jaw muscles become paralyzed, the tongue hangs out, and saliva flows from the mouth in copious quantities. The eyes are dull and the pupils dilated, and the face has a peculiar cunning and troubled expression. Gradually as the paralysis spreads the animal becomes emaciated, the exhaustion increases, and the patient dies in convulsions. During the excitable stage the temperature rises one to three degrees, but later during the paralytic stage the temperature is usually below normal. The pulse is rapid according to Blaine.(13) Courmont and Lesieur(14) found that the polymorphonuclear leucocytes are gradually increased in the blood to the end of the disease. Mocard(15) states that the urine usually contains sugar.

While this description of the course of the disease in dogs may often be somewhat modified, the same course with slight modifications is seen in other infected animals; such as, cats, horses, cattle, sheep, goats, and swine. The disease has a course of from four to seven days but may last from eleven to thirteen days or in exceptional cases even longer. Artificially infected dogs have been known to recover from the disease, but generally speaking with few exceptions the disease is fatal (Pasteur). Cases where dogs were still alive after persons bitten by them had already died from rabies have been reported by Talko and Johne.(16) Of the untreated persons bitten by rabid dogs, about 16 to 20 per cent die of rabies. According to Babes(17) 60 to 90 per cent die after bites of wolves. In human beings the symptoms of rabies are characterized first by depression, itching in and around the wound, and fever. Soon the patient becomes uneasy, respiratory irregularities develop, swallowing becomes difficult, and there is a distinct aversion for all liquids. Salivation becomes increased, and with the increased reflex excitability that develops attacks of delirium appear. Finally paralysis begins to appear, first involving the muscles of the face, eyes, and tongue; gradually involving the trunk and limbs.

Animals susceptible to rabies.—All mammals are susceptible. Birds have been known to contract the disease. Rabies is common in dogs, wolves, jackals, foxes, and hyenas. According to Rosenau(18) the disease is comparatively rare in cats and skunks. The author, however, has seen in Texas an epidemic of rabies in cats which was rather extensive. The disease is considered to be much less frequent in cattle, horses, swine, goats, and sheep, but it does occur with symptoms quite similar to those found in the dog. Rabies can be transmitted to guinea pigs and rats. Infected birds not infrequently recover, but in those in which the disease is fatal only two or three days elapse between the first symptoms and the development of paralysis and death.

The virus of rabies.—That the filterability of the virus of rabies is beyond question has been amply demonstrated by Remlinger and confirmed by Berterelli and Volpino, by Poor and Steinhardt, and by others. The virus may appear in the saliva five days before the animal shows symptoms and has been demonstrated in the saliva of recovered dogs twenty days later. The virus is found in the tear glands, adrenals, pancreas, spermatic fluid, vitreous humor, urine, lymph, milk, spinal fluid, ventricular fluids, and occasionally in the blood. It is distributed throughout the central nervous system and in the peripheral nerves supplying affected parts of the body.

The virus of rabies is supposed to have been cultivated by Noguchi in 1913.(19) Noguchi placed small pieces of brain tissue from rabid animals with pieces of kidney of healthy rabbits into ascitic fluid. After incubation at 37° C. and after several generations, he found minute pleomorphic bodies which stained red with Giemsa's method. Inoculation of this material into susceptible rabbits produced typical rabies. These experiments have not been confirmed by other investigators. Volpius,(20) as well as Kraus,(21) has observed similar bodies in sterile ascitic fluid and concludes that they are identical with lipoid droplets. The experiments of Pfeiler and Klump,(22) who described the cultivation of rabies virus in peptone containing Martin's broth, have not been confirmed.

Resistance of the virus.—The virus in nerve tissue, when protected from light and dried at 20 to 22° C. dies in from five to eight days. It is destroyed by sunlight in about forty hours when exposed on glass slides in thin layers. It is quite resistant to putrefaction. Glycerin preserves the virus. The virus is resistant to freezing but is destroyed when exposed to 60° C. for one-half hour. Five per cent phenol destroys the virus in about seven days, though it resists 0.5 per cent phenol. Cumming(23) has shown that the virus is destroyed in about three hours by various aldehyde compounds. The virus is destroyed by 0.08 per cent formaldehyde within two hours, and by a 1:1000 solution of bichloride of mercury or iodine within one hour.

Pathology (*cell inclusions; Negri bodies*).—At autopsy, in carnivorous animals, the stomach may be found contracted and empty, or filled with foreign substances; such as, straw, hay, sand, pieces of wood, stones, bones, hair, leather, and feathers. The mucous membrane of the stomach is congested and the rugæ show hæmorrhages and erosions. Foreign bodies may also be found in the œsophagus or the intestines. There may also be present a catarrhal inflammation of the respiratory organs, hyperæmia of the salivary glands, the liver, the spleen, and the kidneys, and an œdematous inflammation of the meninges and of the gray matter of the brain. Schaffer(24) recently studied the changes in the central nervous system in six cases of hydrophobia in man. He found cellular infiltration with either capillary or large hæmorrhages in the segment of the spinal cord where the nerves coming from the place of infection enter, in the perivascular lymph spaces, in the walls of the vessels of the gray matter of the anterior horn, in the vicinity of the central canal, and also along the connective-tissue trabeculæ of the white substance. In the nerve cells he found fibrillation, hyaline and vacuolar degeneration, granular disintegration, and pigmentation atrophy of the cellular bodies. In the medulla, below the floor of the fourth ventricle and around the origin of the twelfth, the tenth, and the seventh pair of nerves, there were found hyperæmia, perivascular cell infiltration, and small hæmorrhages. The nerve cells showed signs of degeneration. Slight hyperæmia and cellular infiltration were found in the brain. Csokor and Dexler(25) attach great importance to small foci of inflammation in the brain and to the perivascular cellular infiltration, but Trolldenier found that these lesions are absent in about 60 per cent of cases and that they may also be found in the nervous system of dogs suffering from distemper. Elsenberg and Kossjokow have demonstrated cellular infiltration of the interacinous connective tissue as well as granular fatty degeneration of the glandular cells of the salivary glands.

The most constant finding in the central nervous system is the presence of Negri bodies. Negri described these bodies in 1903 as small, round, oval, or three-cornered inclusions, measuring about 1 to 27 microns in length and 1.5 to 5 microns in width. These bodies contain in their interior very small, refractile, and sharply outlined granules. Volpino(26) described these bodies as consisting of a hyaline ground substance in which sometimes very small marginal, and, at other times, larger central formations seem to be embedded, which contain very fine ring-, rod-, or dumb-bell-shaped inclosures.

Stained by the Giemsa method these bodies are light blue in contrast to the darker and more violet cell bodies. Other methods of staining are according to the methods of Mann, Van Gieson, and a modification of the latter's method by Williams and Lowden.(27) By the Mann method the nerve cells are stained pale blue and in their cytoplasm the small oval bodies are found stained deep pink. Sections of tissue after fixation* are, according to the Mann method, stained for from twelve to twenty-four hours in a solution prepared as follows:

	Cubic centimeters.
Methylene blue (Grübler 00), 1 per cent	35
Eosin (Grübler BA), 1 per cent	35
Distilled water	100

Differentiation is then made in:

Absolute alcohol	30
Sodium hydrate, 1 per cent in absolute alcohol	5

A rapid method for demonstrating Negri bodies in smears of brain tissue is very convenient for diagnosis. Van Gieson(28) recommends the following procedure: A pinhead-sized piece of tissue taken from the hippocampus major is placed upon a clean glass slide. A second slide is placed over the first and the two pressed firmly together until the tissue is flattened out into a thin layer. The two slides are then drawn apart in a sliding motion which further distributes and thins out the material into very thin smears. After fixation in methyl alcohol the smears may be stained by the Giemsa method or by the method recommended by Van Gieson as follows:

Distilled water, cubic centimeters	10
Saturated alcoholic solution of rosanilin violet, drops	2
Saturated aqueous solution of methylene blue, drop	1

This method demonstrates the inner structure of the Negri bodies besides giving excellent contrast.

The assumption that Negri bodies represent products of degeneration seems to be in error when one considers their complicated structure and the fact that they are specific for rabies.

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* Fixation of a small piece of tissue usually taken from the center of the hippocampus major (cornu ammonis) is carried out in Zenker's fluid for about twelve hours prior to dehydration and embedding in paraffin.

However, there is just doubt as to their parasitic nature. Negri bodies are generally lacking in the initial stages of the disease and in animals infected experimentally with fixed virus. It is possible, however, that the virus consists of extremely minute bodies of this nature which are invisible ordinarily and only develop within the central nervous system of infected animals.

In 1906 Williams and Lowden(29) described certain cyclic changes in Negri bodies and concluded that they were a type of Sporozoa and the actual cause of the disease. These authors suggested the name *Neurocytes hydrophobiæ* for these structures. During the same year Calkins(30) supported these conclusions. This concept was further substantiated by Kelser(31) in his work published in 1924.

Negri(32) has described a stage in the evolution of these bodies where the formations contain a nucleus of homogeneous substance which breaks up into very minute chromatin granules. In this way small corpuscles are formed which later grow into new independent bodies. Koch and Rissling(33) and Babes(34) have described coccilike formations about 1 micron in size surrounded by a light capsule in sections of the cornu ammonis and in the degenerated ganglion cells. These findings lend weight to Negri's concept. Prowazek(35) considers the capsulated small formations as protozoans and classifies them with his group "Chlamydozoa." The true nature and significance of these bodies will await further experimentation, though the protozoan theory is apparently gaining more and more ground as new facts are being brought to light.

Immunity.—That there is a certain degree of natural immunity to rabies in some animals is well known. Even in markedly susceptible animals such as the dog there has appeared some evidence of immunity. It is a well-recognized fact that once symptoms of the disease have developed it is impossible by treatment to alter the fatal course of the disease. Yet Galtier(36) observed intermittent rabies in two dogs, and Lignieres(37) in one dog. After the first attack a marked improvement followed, in thirty-six, twenty-seven, and twenty-eight days, respectively; however, an aggravation with fatal termination resulted. Recovery of dogs from rabies has already been noted. There also seems to exist some degree of immunity in man against the virus of rabies as evidenced by the fact that of all persons bitten by rabid dogs, only 16 to 20 per cent of cases not receiving any protective treatment die of the disease.

In general, however, considering the susceptible forms of life it may be said that comparatively little immunity exists, and protective immunization in inoculated cases, whether man or animals, is highly important.

TABLE 4.—*The treatment of rabies at the New York Department of Health.*

Days of treament.	Mild cases.	Dose.	Medium cases.	Dose.	Severe cases.	Dose.
	Days of drying.		Days of drying.		Days of drying.	
		cc.		cc.		cc.
1	14+13	4	10	4	A.M.10+9P.M. 10+9	4
2	12+11	4	9	4	A.M. 8+7P.M. 8+7	4
3	10+9	4	9	4	6	4
4	8+7	4	8+7	4	4	4
5	6	2	6	2	3	2
6	5	2	5	2	4	2
7	4	2	4	2	3	2
8	3	2	3	2	2	2
9	5	2	2	2	4	2
10	4	2	5	2	1	2
11	3	2	4	2	4	2
12	5	2	3	2	3	2
13	4	2	2	2	2	2
14	3	2	4	2	4	2
15	5	2	3	2	1	2
16	4	2	2	2	4	2
17			4	2	3	2
18			3	2	2	2
19			2	2	4	2
20					3	2
21					2	2
22					4	2
23					3	2
24					2	2
25					4	2
26					3	2

According to Horowitz-Wiassowa(38) the serum of rabies-infected or immunized rabbits gives fixation with the rabies virus (fixed as well as street). Fixation was not obtained with the serum of persons subjected to preventive treatment against rabies. Kraus and Michalka(39) suggest the use of boiled antigens (Koktoantigens) in tests in cases of suspected rabies. The same authors(40) find that the complement-fixation reaction, using both Koktoantigen and glycerol extract of the brain of rabbits infected with rabies, together with immune serums, is as specific as it has been with other filterable viruses reported by Kraus, Takaki, and others. According to Nedrigailoff and

Sawtschenko(41) the serums of men and animals that die of rabies give a specific reaction with an antigen prepared from the salivary glands of a rabid animal.

Artificial immunization can be carried out according to several methods, details of which can be found in standard texts on hygiene and bacteriology. Those that have been used are as follows:

1. Protective vaccination with dried substance of spinal cord. Pasteur's method.
2. Protective vaccination with diluted virus. Högyes's method.
3. Protective vaccination with desiccated-at-low-temperature virus. Harris's method.
4. Protective vaccination with dialyzed virus. Cuming's method.
5. Protective vaccination by intravenous injection of cerebral substance (for animals). Galtier's method. (Considered unsatisfactory.)
6. Serum immunization and vaccination. Babes and Lepp's method.
7. Protective vaccination with phenol-glycerinized virus. Umeno and Doi's method.

Of all the methods of protective vaccination mentioned above the Pasteur method is still in most general use throughout the world. There are many modifications of this method, but the fundamentals rest upon the original Pasteur method. It is impossible to compare results with the various methods cited although each method has its adherents.

Artificial immunity appears in about two weeks after treatment and lasts for a varying period of time. In general it is thought that immunity conferred in this way lasts about a year, although it is known that in some cases protection fails entirely and a small percentage of treated cases die of rabies. The immunity produced is not of the nature of an antitoxin. The serum of an immunized animal is verucidal for the virus of rabies in vitro, the immune bodies appearing in the blood about twenty days following the last injection of vaccine.

Control of rabies.—Various control measures are advocated to keep this disease within bounds or totally to eradicate it. These measures are discussed in detail in standard texts on preventive medicine. In general they consist of laws regulating importation of dogs, muzzling, quarantine, licensing, education and responsibility, and immunization. While protective vaccination of dogs (the most important reservoir of the virus of rabies) is just now becoming general throughout the world, this measure bids fair to alter most favorably the picture of the disease as a public-health problem.

BIBLIOGRAPHY

1. Galtier, Comp. rend. des sceances de l'Acad. des Sci. (1879).
2. Pasteur, Comp. rend. des sceances de l'Acad. des Sci. (1881, 1882, 1884, 1885, 1886).
3. Pasteur, Comp. rend. des sceances de l'Acad. des Sci. (1881, 1882, 1884, 1885, 1886).
4. Remlinger, Ann. de l'Inst. Pasteur **17** (1903) 834.
   Remlinger and Riffat-Bey, Soc. Biol. (July 11, 1903).
5. Bertarelli and Volpino, Central bl. f. Bakteriol., 1te. Abt., Orig. **37** (1904) 51.
6. Poor and Steinhardt, Journ. Infec. Dis. **12** (1913) 202.
7. Negri, Zeitschrift für Hygiene und Infektionskrankheiten **43** (1903) 507.
8. Volpino, Giorn. d. r. Accad. d. med. Torino **14** (1903) 228.
9. D'Amato, Riforma med. **20** (1904) 617.
10. Bertarelli, Centralbl. f. Bakteriol. **37** (1906) 556.
11. Bosc, Compt. rend. de la Soc. de Biol. **55** (1903) 1436.
12. Poor, Proc. N. Y. Path. Soc. (1904) Journ. Infec. Dis. **3** (1906) 101.
13. Blaine, Quoted from Hutyra and Marek, Am. ed. by Mohler and Eichhorn, 3d ed. (1926) 577.
14. Courmont and Lesieur, loc. cit.
15. Nocard, loc. cit.
16. Talko and Johne, op. cit. 582.
17. Babes, op. cit. 596.
18. Rosenau, Preventive Medicine and Hygiene, Appleton, 5th ed., p. 33.
19. Noguchi, Journ. Exp. Med. **17** (1913) 29.
20. Volpius, Quoted from Hutyra and Marek, Am. ed. by Mohler and Eichhorn, 3d ed. (1926) 565.
21. Kraus, loc. cit.
22. Pfeiler and Klump, loc. cit.
23. Cumming, Journ. Infec. Dis. **14** (1914) 33.
24. Schaffer, quoted from Hutyra and Marek, Am. ed. by Mohler and Eichhorn, 3d ed. (1926) 572.
25. Csokor and Dexler, loc. cit.
26. Volpino, op. cit. 564.
27. Williams and Lowden, Journ. Infec. Dis. **3** (1906) 452.
28. Van Gieson, Proc. N. Y. Pathol. Soc., N. S. **4** (1906); Centralb. f. Bakt. **43** (1906) 205.
29. Williams and Lowden, loc. cit.
30. Calkins, Discussion, Proc. N. Y. Pathol. Soc., N. S. **6** (1906).
31. Kelser, Journ. A. V. M. A. **64** (1924) No. 6.
32. Negri, Z. f. Hyg. **63** (1909) 421.
33. Koch and Rissling, Z. f. Hyg. **65** (1910) 85.
34. Babes, Tr. de la Rage, Paris (1912).
35. Prowazek, Quoted from Hutyra and Marek, Am. ed. by Mohler and Eichhorn, 3d ed. (1926) 565.
36. Galtier, J. vet. **8** (1904) 274–330.
37. Liginieres, Quoted from Hutyra and Marek, Am. ed. by Mohler and Eichhorn, 3d ed. (1926) 578.
38. Horowitz-Wiassowa, Centralbl. f. Bakteriol. **98** (1926) 216.
39. Kraus and Michalka, op. cit. 504

40. KRAUS and MICHALKA, Ztschr. f. Immunitstatsforsch. u. exper. Therap. 47 (1926) 504.
41. NEDRIGAILOFF and SAWTSCHENKO, op. cit. 596.

PSEUDORABIES: INFECTIOUS BULBAR PARALYSIS

Definition.—Infectious bulbar paralysis, or pseudorabies, is an acute infection of the central nervous system of dogs, cats, and cattle which is characterized by paralysis of the pharynx and marked painful pruritis on various parts of the body. Unlike rabies proper the virus is absent from the saliva but present in the blood stream. The disease is highly fatal, death of affected animals resulting within thirty-six to fifty hours after infection takes place.

History.—Aujeszky(1) in 1902 first observed this disease in rabbits following the injection of medullary substance from a cow and a dog both of which had died with symptoms suspicious of rabies. Marek(2) found this condition to be present in one hundred eighteen cats and twenty-nine dogs in a clinic at Budapest from 1902 to 1908. The disease is said to have been observed in certain parts of Brazil, where it is known as "peste de cocar" (itch plague); it has been seen in Russia and possibly in Germany.

Distribution.—Infectious bulbar paralysis has been present chiefly in Hungary but has been reported in Brazil, in the Dorogobusch district of Russia, and possibly only once by Bass(3) in Germany.

Incubation period.—The incubation period in infectious bulbar paralysis varies from twenty hours to ten days following artificial infection. Usually following inoculation rabbits die within twelve to thirty-six hours. The nature and mode of the natural infection are little known.

Symptoms.—The symptoms of infectious bulbar paralysis are described by Hutyra and Marek as follows:

> Dogs and cats become apathetic, change their resting places often and sit crouched up; cats meow and yawn, sometimes painfully. There is profuse salivation; inappetence is present from the onset of the disease with frequent vomiting, and constant symptoms of paralysis. In about half of the cases the patients manifest severe itching on any part of the head, which they scratch with the fore paws, or rub against some object, sometimes so severely that inside of several hours extensive abrasions or even deeper injuries result. In other cases this symptom is absent, but the groaning or painful meowing indicates that the animals are in pain. With rare exceptions the examination of the nervous system shows paralysis of the pharynx and pruritus, also an uneven dilation of the pupils

associated with a first increased reflex irritability, later diminished pupillary reflex, muscular sensibility, and superficial and deep reflexes. In most cases periodical twitching may be observed in the flexor muscles of the head and neck, sometimes also the muscles of the lip. The respiration is markedly labored, the temperature is normal or only slightly elevated and the patients succumb almost exclusively inside of 24 to 36 hours. In cattle a persistent rubbing of the muzzle or of other parts of the face is observed, which results in more or less extensive bloody areas, denuded of hair and surrounded by edematous swellings. At the same time the animals bellow loudly, and kick spasmodically with their hind legs. In the meantime a pronounced swelling and weakness of the hind quarters develop while the appetite is for a time normal. Finally the disease terminates in death after 12 to 36 hours.

Symptomatically then the disease is quite different from rabies proper.

Animals susceptible to the virus of pseudorabies.—Besides cattle, dogs, and cats, which are all markedly susceptible, the disease may be produced in laboratory animals such as rabbits, guinea pigs, rats, and mice. Sheep are easily infected, while the ass is less susceptible, and horses can be infected only with difficulty. Fowls are not susceptible. In man the infection, according to Ratz,(4) causes only a local inflammatory swelling which persists for several days, then disappears.

The virus of pseudorabies.—The virus is present in the tissue fluids at the point of inoculation, in the blood stream, and in the central nervous system. It is not present in the saliva, bile, or urine. One one-thousandth cubic centimeter of blood from an affected animal is sufficient to induce the infection in a susceptible animal. The virus is filterable through coarse porcelain filters which retain bacteria. All attempts to cultivate the virus have failed. The virus is preserved in 50 per cent glycerin as are many of the filterable viruses. It is rendered avirulent by drying from three to six days. Heating at from 55 to 60° C. destroys the virus in about thirty-five minutes, while 80° C. destroys it within three minutes. The virus is also destroyed within a few minutes by 0.5 per cent hydrochloric acid, by 1 per cent corrosive sublimate, or by 5 per cent phenol (Schmiedhoffer(5)). According to Zwick and Zeller(6) the virus is destroyed by decomposition in eleven days at the longest.

The disease may be produced artificially in cats, dogs, and rodents by feeding infected tissue. The possibility that domestic animals may be infected by diseased rats or mice has been suggested by Hutyra, while Kolonits(7) has suggested the possibility that the disease is transmitted by the spines of plants and rough fodder that have been contaminated by an infected animal.

Immunity.—Little is known regarding immunity in pseudorabies. The disease is practically 100 per cent fatal, although recovery in a cat has been observed. (Hutyra and Marek.)

Pathology.—Autopsy of animals dead of the disease reveals hyperæmia and small hæmorrhagic extravasations in the meninges. There are also found inflammatory lesions in the parts of the body that were painful and pruritic during the life of the animal.

BIBLIOGRAPHY

1. Aujeszky, Cbl. f. Bakt. **32** (1902) 353.
2. Marek, Jahresber. d. Hochschule Budapest (1902–03, 1908–09).
3. Bass, D. t. W. (1910) 396.
4. Ratz, A. L. (1910) 297.
5. Schmiedhoffer, Z. f. Infkr. **8** (1910) 383.
6. Zwick and Zeller, Arb. d. G. A. **36** (1911) 382.
7. Kolonits, A. L. (1912) 615.

HERPES SIMPLEX

Fever blisters; Herpes (French); Blaschenflechte (German)

Definition.—Herpes simplex is an acute eruptive disease of the skin and the mucous membranes, and usually occurs upon the face and genitalia. It is characterized by a vesicular eruption, the vesicles appearing in groups upon a mild inflammatory base, and accompanied by sensations of heat and burning. The disease is caused by a filterable virus, the manifestations of which are influenced by a number of factors; such as, local irritation, exposure to the sun's rays, drafts of cold air, traumatism, and various constitutional and infectious diseases. Herpes is designated, according to location and conditions under which it occurs, as herpes febrilis, herpes labialis, cold sores, herpes genitalis, herpes preputialis, and generalized herpes.

Occurrence of herpes.—This disease has been known since ancient times. It occurs in connection with a number of infectious diseases. According to various dermatologists the disease is said to be present in about 40 per cent of cases suffering with cerebrospinal meningitis, lobar pneumonia, and malaria; in about 6 per cent of scarlet-fever patients; 6 per cent of influenza cases; 5 per cent of typhus cases; and in a comparatively small number of patients having diphtheria, typhoid fever, relapsing fever, and smallpox. The affection is common in prostitutes, and according to Unna it occurs, in Hamburg, in about

25 per cent of these women. The disease frequently manifests itself in women about the time of the menstrual period. It occurs in connection with the acute coryzas and various gastric and intestinal conditions. Focal infections in the mouth may also be accompanied with herpetic lesions. In other cases herpes has apparently been related to the ingestion of certain foods. In many individuals the affection tends to be recurrent and the lesions may appear in the same area over a long period of time. These recurrent lesions are particularly noted upon the face, about the buttocks, and upon the genitalia.

Symptoms.—Herpes may involve any part of the cutaneous surface but is more commonly found on the face and about the genitalia. The affection is self-limited and usually terminates within a week or ten days. More often the lesions are few and localized, but occasional generalized distribution occurs. Mild constitutional symptoms may be present, and the part affected is subject to sensations of heat and burning. There may be present some slight elevation in temperature, and chilliness, and on this account the disease is frequently referred to as "fever blisters." In few cases the lesions appear upon the mucous membranes of the tongue, cheek, pharynx, and larynx. Occassionally the vesicular patches are fairly large, and may coalesce, becoming cloudy and purulent. After a few days the vesicles dry and yellowish crusts are formed which drop off within a short time leaving a brownish red stain which soon disappears.

Epidemics of herpes have been reported by Savage(1) and by Seaton.(2) Both of these epidemics occurred in schools for boys and the cases were characterized by sudden onset, elevation of temperature, chills, headache, nausea and vomiting, and herpetic eruption chiefly on the lips and other parts of the face. The occurrence of the disease in epidemic form is strong indication that the disease is caused by an infectious agent.

The virus.—In 1913 Gruter(3) demonstrated that the inoculation of the rabbit's cornea with scrapings from lesions of dendritic keratitis produces in rabbits an inflammatory process which is transmissible from animal to animal. This observation was confirmed latter by both Lowenstein(4) and by Luger and Lauda,(5) and these authors further demonstrated that precisely the same process can be induced in rabbits by inoculating the rabbit's cornea with the fluid from herpes vesicles. Lowenstein believed that the infectious agent existing in the fluid of the herpes vesicle is filterable, and this belief was fully established in the later work of Luger and Lauda, Blanc and Caminopetros,(6) and Levaditi, Harvier, and Nicolau.(7)

Lipschütz(8) in 1921 was the first to demonstrate cell inclusions in the lesions of herpes simplex, herpes genitalis, and herpes zoster. These inclusions are without doubt specific as indicated by the fact that they are also found in the epithelial cells of the inoculated rabbit's cornea. These bodies are found only in the actual seat of the lesion, and in herpes simplex (febrilis) and herpes genitalis they have been found to appear in serial inoculations. Unlike most of the intracellular inclusions described in connection with other filterable virus diseases, the inclusions in herpes are found almost exclusively within the nucleus and not in the cytoplasm of the cell.

More will be said of the inclusion bodies in Chapter XVII. For the present we may state that the exact nature of these bodies is unknown. However, it is well known that such bodies are frequently met with in connection with several of the diseases caused by agents of the filterable virus group, and their presence is now considered as prima-facie evidence of the activity of a filterable virus. Indeed, in the case of the virus of herpes simplex and of herpes genitalis, the filterability of the viruses has been demonstrated.

As indicated before there are many other factors which apparently contribute to the development of herpes lesions. It is well known that in certain individuals herpes has never occurred during an entire life time. It is evident from the beginning, then, that there exist varying degrees of susceptibility and resistance to this disease. Irritation, trauma, diet, emotional disturbances, and infectious diseases (for example, meningitis, pneumonia, and malaria) may all contribute to the development of the disease. In certain men the simple act of coitus is sufficient to bring on an attack of the affection. Its occurrence in prostitutes and in women about the time of the menstrual period has already been mentioned. We suggest that all of these influences are only contributing in nature and none of them is related to the real cause of the disease. Herpes is an infectious disease, caused by a filterable virus. The virus of herpes is apparently widely distributed. Similar viruses have been isolated from the saliva and from the nasal secretions. Presumably there are untold numbers of healthy carriers of the virus. The extraneous factors mentioned above may contribute to the development of lesions by the herpes virus already present, latent in the individual, by lowering the general resistance of the individual to the virus. We have suggested this concept in another publication.(9)

Goodpasture and Teague(10) have shown that the virus of herpes passes up the axis cylinder of the nerve from its point of entrance to the spinal ganglia and the central nervous system in rabbits. It has, of course, been known for years through the work of Howard(11) and Mallory and Wright(12) that degenerative and inflammatory changes are present in the ganglionic centers supplying areas upon the face affected by herpes in cases occurring with pneumonia and cerebrospinal meningitis.

It appears then that the virus of herpes reaches the surface by way of the nerves. If the virus passes up the nerve trunk there is no reason to suppose that it cannot pass down the nerve to the cutaneous area supplied by that nerve. Does the virus remain latent then in the tissues of man, and more particularly in the nerve tissues such as the spinal ganglia? The fact that recurrent herpes is frequently brought on as a result of great emotional or nervous stress favors this idea somewhat.

The changes found in the ganglionic centers have been thought by some authorities to be of toxic origin. While no toxin has as yet been demonstrated for the herpes virus it is logical to suppose, from our ordinary concept of the mechanism of infection, that such degenerative and inflammatory changes are caused by a toxin elaborated by the virus. It must be left to future investigations to determine this question.

Within recent years the herpes virus has received a great deal of attention, since several filterable viruses have been demonstrated in the brain substance and spinal fluids from cases of epidemic encephalitis that are apparently identical with or, at least, closely related to the herpes virus. Doerr and Vöchting(13) have shown that the herpes virus is capable of inducing an encephalitis in rabbits when injected intraocularly. Since the work of Doerr and Vöchting numerous investigators have shown that encephalitis in rabbits regularly follows the subdural injection of the herpes virus. Strains from herpes simplex (febrilis) and herpes genitalis are practically all found to be potent in this respect. So far no one has succeeded, in unquestioned experiments, in producing encephalitis in rabbits following the subdural inoculation of fluid from the vesicles of herpes zoster. Cole and Kuttner(14) failed in this respect with material taken from nine cases, and McKinley and Holden(15) failed in three instances. However, there is strong evidence to suppose that herpes zoster is also a virus disease, but so far the hypothetical virus has remained undemonstrated.

To summarize we may conclude that herpes simplex and herpes genitalis are caused by a filterable virus. Cultivation experiments with this virus have so far resulted in only discouraging results. By several methods Le Fèvre and McKinley(16) failed entirely in the cultivation of this virus. Parker and Nye(17) have reported more-encouraging results, but their work cannot be fully accepted at present as having demonstrated the artificial cultivation of this agent. Herpes is a common affection, and this indicates that the virus is wide spread. We propose the theory that the herpes virus is present and latent in the tissues and secretions of man who serves as its reservoir. Under favorable conditions acompanied by a lowering of the individual resistance, brought about by a variety of influences, the virus becomes active and manifests itself in cutaneous lesions. The relation of this virus to epidemic encephalitis will be discussed in the following section.

EPIDEMIC ENCEPHALITIS: LETHARGIC ENCEPHALITIS

Definition.—Epidemic encephalitis is an infectious disease of protean manifestations. Encephalitis means brain inflammation. Epidemic encephalitis is an inflammation chiefly of the central nervous system and is characterized in most cases by lethargy, paralysis of the cranial nerves, and in some cases there is spinal- and peripheral-nerve involvement. Not all cases of epidemic encephalitis manifest lethargy for in some patients, depending upon the location and severity of the involvement, marked excitement may be a predominating symptom. This disease is also sometimes referred to as epidemic stupor, infective encephalitis, epidemic polioencephalitis, and sleeping sickness (European).

History and distribution.—Little is known regarding the early history of epidemic encephalitis. Early after 1700 a disease broke out in parts of Germany which epidemiologists believe may have been related to epidemic encephalitis. Again in 1890 in parts of southern Europe there appeared a peculiar disease, designated "nona" at the time, which may have been related to encephalitis lethargica. From the records it is impossible to state definitely whether or not either of these diseases was related to epidemic encephalitis.

In 1917 Economo(18) described, under the name of encephalitis lethargica, a disease which he observed in eleven cases in Vienna. These cases had occurred during the winter of 1916–17. During the spring of 1918 the disease began to appear in various parts of England, and toward the end of 1918 the first indications of the disease were noted in the United States. The early cases of encephalitis lethargica were apparently regarded as brain inflammations due to botulism. The disease was characterized by drowsiness passing into lethargy, a progressive muscular weakness, and opthalmoplegia. Often the first symptoms noted were a slight indisposition, mild indefinite muscular pain, and a feeling of drowsiness. Both males and females are affected and in about equal proportions. Most of the cases have appeared in individuals over 20 years of age. This is quite the opposite of poliomyelitis which occurs chiefly in children. While most cases of epidemic encephalitis occur in individuals in their twenties or thirties, the disease also occurs in later years after the age of 50 and in children.

Since 1917 epidemic encephalitis has spread to all parts of the world. In England alone thousands of cases are now being reported each year. During 1924 England reported over five thousand cases of this disease according to the Epidemiological Report of the Health Section of the League of Nations. This report states that in most countries epidemic encephalitis is decreasing at the present time. England, for example, has reported only 2,267 cases for the year 1926 with 1,325 deaths from this disease. In England the disease is more prevalent in urban than in rural districts. In New York City cases of epidemic encephalitis are not at all uncommon, and the same is true of other large cities in the United States. The disease is not limited to cities, however, and is frequently met with in rural communities. The disease has a high mortality and shows a predilection for the winter months.

The only epidemics of a similar disease, which have been mentioned above, occurred in connection with or following epidemics of influenza. In 1918 when epidemic encephalitis began its march over the world it was thought that the disease was related to influenza and perhaps resulted as a sequela, in certain cases, as a result of the same infection. Except for epidemiological data there is no evidence that epidemic encephalitis is related to epidemic influenza except as a concurrent infection. It is true that in England the incidence of epidemic encephalitis is highest in the north of England, decreasing through the south, and lowest in Wales. Also influenza is more prevalent in the north of England, but the mortality of encephalitis is highest in urban communities while influenza has a greater mortality in rural districts.

Symptoms.—The usual picture of epidemic encephalitis is lethargy, associated with third-nerve and facial paralysis, and weakness in the lower extremities. There are many clinical forms of the disease which depend upon the location and degree of involvement of the central nervous system. Some of the different types of the disease have been described as follows: Cases exhibiting general manifestations of the disease but no localizing signs; cases with facial paralysis; cases with third-nerve paralysis; cases with spinal-cord manifestations; cases with polyneuritic symptoms; cases showing periods of great excitement; and mild cases of the abortive type having only transient manifestations. All degrees, variations, and combinations of the above types have been met with.

Prodromal symptoms may be present or absent. If present they may be noted from a few hours to a few weeks and are characterized chiefly by headache, diffuse pains, lethargy or drowsiness, stiffness of the back or of one or more limbs, conjunctivitis, and vertigo.

Lethargy, a prominent symptom of the disease, occurs in about 80 per cent of the cases. It comes on as a rule very gradually but may occur suddenly. It may develop gradually from a slight drowsiness to a stupor from which the patient can be aroused and then pass on into a deeper stupor or coma. Ocular palsies, with double ptosis and diplopia occur early. The temperature ranges from 100 to 104° F. It usually lasts but a few days and may then disappear only to recur, in some cases within a few days.

The disease is slowly progressive. The patient takes on an apathetic look, the pupils may be dilated and unequal with complete third-nerve paralysis. The face becomes smooth and expressionless, the muscles being moved with great difficulty. Catalepsy is not uncommon. The patient may become delirious and develop a violent mania. The memory is lost gradually, and the patient cannot remember one moment what he has been told a few moments before. Aroused he may answer simple questions intelligently. Tremors and choreiform movements may occur in some cases and persist for some time.

Sensory disturbances, such as hyperæsthesia, may occur but are rare. Paralysis of the arms and the legs may occur. The reflexes may or may not be normal. Some cases exhibit paraplegia. Other cases may show bulbar features and signs of polyneuritis. Signs of meningeal involvement are rare. The cerebrospinal fluid is clear, may show 10 to 20 cells per cubic millimeter, and in some cases a large increase. Both mononuclears and polynuclears are found. The duration of the disease is variable. It may last weeks, months, or years. As a rule its duration ranges from two to twelve weeks. Second attacks occur and apparently, in many cases, are more severe than the first illness. These are probably not reinfections but a flaring up of the old process when conditions are favorable.

There are other forms of encephalitis from which epidemic encephalitis must be differentiated along with several other conditions that are similar in some respects. Acute encephalitis may result from a number of causes such as trauma, intoxications (alcohol, food poisoning, and gas poisoning), following acute infections, and as a form of polio-myelo-encephalitis. The general symptoms are headache, somnolence, coma, delirium, nausea and vomiting, etc. Cases may occur following certain fevers, such as influenza and typhoid.

The virus of epidemic encephalitis.—The virus of epidemic encephalitis is unknown. That the disease is infectious and communicable is agreed by most authorities. What the infectious agent is or how it is communicated from individual to individual is unknown at present. In 1919 Loewe and Strauss(19) described an organism as the cause of epidemic encephalitis which they obtained from the brain substance and nasopharyngeal washings from cases of this disease and claimed to have reproduced the disease in rabbits and monkeys with this material. Later they cultivated their organism and reproduced the disease with the cultivated microörganism. These experiments have been open to grave doubt and have not been confirmed.

In 1922 Levaditi and Harvier(7) described a filterable virus which they believed to be the cause of epidemic encephalitis. In reality there were two strains of this virus obtained from cases of this disease. The first and most virulent virus was obtained from the brain substance of a case of encephalitis, while the second and weaker virus was obtained from nasal secretions. Flexner (20) has pointed out that "examination of Levaditi's reports indicates that from among inoculations made with thirty separate sets of specimens from cases of epidemic encephalitis, he succeeded in establishing in rabbits only one active virus" and that his conclusions are "based really on a single unequivocal experimental result." Further, it is now well known that the case of encephalitis from which Levaditi obtained his first virus possessed a herpes infection over the entire right side of the face, and subsequent experiments with the Levaditi virus indicates that this virus is identical with known herpes strains or closely allied to them.

Other filterable viruses have been obtained from the brain substance and cerebrospinal fluid from cases of epidemic encephalitis. There are the so-called "Basel" viruses. The first of these was obtained by Doerr and Schnabel (21) from the cerebrospinal fluid of a patient having encephalitis lethargica. The second strain was obtained by Doerr and Berger (22) from the brain substance from a typical case of epidemic encephalitis. The third strain was derived from another case of epidemic encephalitis from the brain substance by Doerr and Berger. Zdansky (23) found that the brain lesions produced with the Basel strains II and III were identical with those produced in rabbits with the Levaditi so-called encephalitis virus and also with the herpes virus.

The "Berlin" virus was described by Schnabel (24) and was obtained from the cerebrospinal fluid of an acute case of epidemic encephalitis. Cross-immunity experiments with this virus and a known herpes virus were found to be positive.

The so-called "Wien" virus was isolated by Luger and Lauda (5) and was obtained from the cerebrospinal fluid and brain substance from a case of epidemic encephalitis. This strain first exhibited an incubation period of eighteen days, which gradually became shorter with animal passage. Symptomatically in rabbits the disease produced by this virus is typical of that induced in rabbits following inoculation with herpes virus. These authors also studied a virus described by Koritschoner; (25) it was derived from the brain substance of a patient who had been bitten by a supposedly rabid dog. The patient died and a post-mortem examination revealed a myeloencephalitis. Inoculation of brain material from this case into two rabbits and two dogs resulted within a few days in paralysis and lethargy. Luger and Lauda believe that the Koritschoner virus is endowed with herpetiform properties, but Doerr and Zdansky(23) identify it with the virus of pseudolyssa or infectious bulbar paralysis. These authors base their conclusions upon its transmissibility to dogs and the absence of Negri bodies in the brains of such infected animals.

Bessemans and van Boeckel(26) experimented with material from five brains, twenty cerebrospinal fluids, and five nasopharyngeal washings from several cases of epidemic encephalitis. Sixty-one rabbits and thirty guinea pigs were inoculated with these materials. Only seven of these rabbits showed symptoms and lesions, and only two guinea pigs showed lesions but no symptoms. Symptomatically these animals exhibited pictures typical of those due to herpetic infection. The lesions consisted of diffuse infiltration of the brain substance with mononuclear cells and in one case perivascular infiltration. This virus possessed only a moderate virulence and was soon lost entirely. In regard to this virus Da Fano(27) states that—

> these experiments seem to indicate the possible occurrence of weak encephalitis strains endowed with properties similar to those of likewise weak or somewhat anomalous herpetic viruses. This supposition is supported by the report of Netter, Cesari and Durand, who claimed to have recovered an apparently weak strain from the brain of a case of lethargic encephalitis 15 months after onset. This virus was regularly active when prepared with the brain substance of successfully inoculated animals, while the salivary secretion of the infected animals was uniformly virulent. Also Sicard, Paraf, and Laplane claim to have isolated an apparently weak encephalitis strain from a case of post-encephalitis Parkinsonism.

In addition to the above viruses that have been obtained, Perdrau(28) in England has apparently had unusual success in isolating viruses from the brain substance from cases of epidemic encephalitis which he believes possess herpetiform properties.

As will be noted the various viruses described above, with the exception of the virus of Loewe and Strauss which is no longer taken seriously, have been obtained from cases of epidemic encephalitis in Europe. Flexner and Amoss(29) and others in the United States have been uniformly unsuccessful in isolating a single virus from cases of this disease. Flexner(20) in 1923 reported transmitting a disease to rabbits with the cerebrospinal fluid from a patient with neurosyphilis, and from these rabbits he obtained a strain of herpes now known as the "Beckley" strain. In this case there was no question of encephalitis, but it serves to illustrate that the herpes virus may occur in the spinal fluid of patients affected with some other disease process.

General opinion is that the various viruses described by European investigators as obtained from cases of epidemic encephalitis are identical with the herpes virus or that they are closely related. Doerr and Schnabel and later Levaditi, Harvier, and Nicolau all found that cross immunity experiments with Levaditi's encephalitis virus and the herpes virus are positive. They later also demonstrated that the Levaditi encephalitis virus produces an herpetiform eruption when inoculated on the skin of a rabbit. Levaditi and his associates, then, consider that the virus of encephalitis and herpes are identical, varying only in degree of virulence. Doerr(30) also leans toward this concept and believes that the herpes virus is related etiologically to epidemic encephalitis.

TABLE 5.—*Active immunization with herpes virus.*

[Dilutions in order of 1 : 1000, 1 : 100, 1 : 10 concentrated.]

Rabbits.	Number and amount of injections.		Period of immunization.		Test, subdural, fourteen days after last injection.	Result.
	Serum virus mixture.	Fresh virus in saline solution.	Total time.	Intervals between injections.		
			Days.	*Days.*		
1-6	Six of 1 cc	Four of 1 cc	37	3-4	0.2 cc.H5318	Survived.
7-10	Four of 1 cc	do	26	3-4	0.2 cc.H4953	Do.
11					0.2 cc.H5318	Died with encephalitis after four days.
12					0.2 cc.H4953	Do.

TABLE 6.—*Passive immunity produced in rabbits with pooled immune serum.*

Rabbits.	Intravenous injection of immune serum.	Subdural test three weeks after last injection.	Result.
1-5	Three injections of 5 cc. each	0.2 cc. virus H5348 [b]	Survived 32 days.
[a] 6		do	Died in 4 days.
[a] 7		do	Died in 6 days.

[a] Central.

[b] The animals again received subdural injections thirty-two days later and all died with typical encephalitis within from three to six days.

Discussing this question in 1924 Parker(31) says:

> There is one fact that would seem to establish definitely the lack of identity between the virus of herpes and that of encephalitis lethargica;

namely, the occurence of the intranuclear bodies characteristic of herpes in the former and the absence of such bodies in the latter. Lipschütz described certain intranuclear, acidophilic bodies that occur, especially in the epithelial cells, in herpes in human beings and in experimental herpes of the rabbit's cornea. These bodies are perfectly definite and their demonstration renders the diagnosis of herpes positive in animals and also in human beings, provided that in the latter varicella can be excluded. Whether they represent the virus or a reaction on the part of the cell about the virus, or merely a reaction or degenerative product of the cells is undetermined, but this does not detract from their diagnostic importance. Since the work of Lipschütz, they have been described by Goodpasture and Teague in the brains of rabbits dying of herpetic infection.

Now if the virus of herpes and that of encephalitis lethargica are identical, these bodies should occur in the brains of human beings dying of encephalitis lethargica. However, to date, no such bodies in these cases have been described. [See Chapter XVII, Da Fano.]

The entire question of the relation of herpes virus to epidemic encephalitis has become a very important one. Indeed, the question has become one of controversy. Investigators are, for the most part, agreed that the cause of encephalitis lethargica is probably a filterable virus. Flexner and his associates have consistently held that the hypothetical virus of epidemic encephalitis remains undiscovered. European investigators have attached more and more etiological significance to the herpes virus as a cause of the disease. Zinsser and Tang(32) have brought further evidence against the herpes virus in its possible relation to epidemic encephalitis. These authors have shown that the serum from encephalitis patients possesses no virucidal effect for the herpes virus in vitro though this property is present in the serum of rabbits immunized with herpes virus.

In a recent publication McKinley and Holden(9) suggest that, in the absence of a definite etiologic agent for encephalitis lethargica and since herpes strains are occasionally met with in the nerve tissue of cases of this disease, it is advisable to retain an open mind on the entire question and attempt in every way possible to prove or disprove any relation that may exist between the herpes virus and encephalitis. The idea of an encephalitis being due to a toxin, as previously suggested by Parker, was again suggested. The occasional presence of the herpes virus in the central nervous system of patients might be explained on the basis of the central nervous system barriers becoming permeable to the virus or its toxin as a result of some other constitutional disorder or infectious process which lowers the resistance of the patient, and consequently of those barriers which normally do not permit the herpes virus or any other virus to gain entrance into the central nervous system. For example, such a hypothesis might explain the occurrence of encephalitis lethargica following in the wake of influenza. If such were the case it might explain the occurrence of those cases in which the herpes virus has been found in the brain. Also, as Perdrau has pointed out, the virus may be more easily obtained during the first few days of illness when the symptoms are acute. Parker suggests that the actual virus of encephalitis may be growing in some other region, such as the gastrointestinal tract, or nasopharynx, without causing local symptoms, but producing a poison that has a marked affinity for the central nervous system. Analogies of this are of course found in tetanus and to some extent in botulism. Such a virus might also be present, latent in the spinal ganglia, and produce its toxin from this focus.

In one of his most recent papers Flexner(33) in commenting upon the ease with which herpes viruses may be implanted in rabbits states:

> On the other hand, it has proven extremely difficult to implant such a virus on rabbits with material taken from cases of epidemic encephalitis in man. The several hundred or more transfers of these materials from man to rabbit have yielded, as Flexner pointed out and Doerr concedes, six successful inoculations at most. The percentage of successes is almost minimal. The matter at issue is the explanation of the disparity, the burden of proof, of course, being placed upon those investigators who would identify the herpes virus with the supposedly microbic incitant of epidemic encephalitis.

This may be taken as the author's attitude in the matter, as far as the herpes virus is concerned.

Up to this point we have omitted entirely from consideration the possibility of the streptococcus as the microbic incitant in encephalitis lethargica. In 1924 Rosenow(34) reported the isolation of a streptococcus from the tonsils, teeth, and nasopharynx of cases of encephalitis and from the brain substance of such cases after death. Evans and Freeman(35) have also described a streptococcus isolated by them from nasal washings, heart blood, and mesencephalon of a patient with epidemic encephalitis. The streptococcus obtained by these authors is similar, according to comparative test, with the streptococci described by Von Wiesner and by Rosenow. This organism, so it is claimed, shows a tendency to elective localization in the brain and is said to produce nervous symptoms in rabbits and monkeys which, in some instances, simulate the disease in man. In another publication Evans(36) reports the isolation of six strains of streptococci from vesciles in cases of herpes, one from the cerebrospinal fluid from a case of syphilis, and one from the brain substance from a case of epidemic encephalitis. Freeman(37) has described the use of an antiserum prepared in horses with the streptococcus which was described earlier by Evans and himself. Administration of specific antistreptococcus serum to cases of encephalitis was controlled by the administration of normal horse serum, antipneumococcus serum, and streptococcus bacterin. Freeman states: "The injection of normal horse serum during one relapse, of antipneumococcus serum during another, and of streptococcus bacterin during a third thus had the same beneficial effect that the encephalitis serum had in the first place." Russell(38) has also described a number of recoveries of cases of encephalitis following the administration of antidiptheritic serum. As pointed out by Freeman such results indicate the need for proper controls in attempting to evaluate the efficacy of specific serum therapy. Certainly these results as reported by him lend little evidence in favor of the streptococcus etiology of this disease. They indicate, however, that nonspecific protein therapy may have some beneficial effects.

In a recent review of the filterable virus diseases MacCallum(39) in speaking of epidemic encephalitis and the experimental work with the various viruses which we have described above states:

> . . . differences between the curves showing the effect of inoculation of encephalitis material on the one hand and of herpes material on the other, into rabbits (Ford and Amoss), make one suspect very strongly that all of the so-called viruses of encephalitis are really accidentally recovered herpes viruses. This seems a far safer conclusion than that ventured by some that the viruses of encephalitis and herpes are identical, and it is preferable to concede that as yet we know nothing of the cause of encephalitis. All of this has been clearly brought out by Flexner.

This is also indicated by the fact that Bastai and Busacca(40) have found the herpes virus in the blood and cerebrospinal fluid of persons subject to herpes but who at the time possess no lesions. In such people slight indisposition frequently brings on an attack of herpes.

This discussion may be taken as representative of the thought of the American school on the subject of the etiology of epidemic encephalitis. With regard to the streptococcus as a possible etiologic incitant, there are few who consider this organism seriously.

Pathology.—The chief changes found in epidemic encephalitis are located in the upper part of the pons and in the basal nuclei and consist of a perivascular infiltration of large and small mononuclear lymphocytes. In some cases the areas of extravascular infiltration form foci which may be seen with the naked eye. The destruction of the ganglion cells as found in poliomyelitis is not common. The spinal-cord lesions are very mild. Changes in the Purkinje cells are noted, but cortical lesions and extensive lesions of the gray matter are not common.

According to McCrae (41) hæmorrhages in the meninges and in the regions of the basal ganglia may be found. Lesions also occur in the cerebellum. Lesions are both nodular and diffuse. The nerve cells show degeneration either localized or general. Thrombosis and necrosis have been noted but are rare. The gray matter at the base of the brain is particularly involved. Altogether, according to this author "the anatomical lesions are like those found in rabies and sleeping sickness." He states further—

> the lethargy may be toxic but is possibly mechanical due to interruption of stimuli in the thalamus, which is frequently involved. The latter changes involve (1) the vessels with hyaline and calcareous degeneration of the media and adventitia especially and particularly in certain areas, especially the basal ganglia, (2) hydrocephalus, chronic or intermittent, from imperfect drainage of the ventricles, and (3) meningeal thickening.

Prevention.—Very little can be said regarding the prevention and control of epidemic encephalitis. That the disease is communicable is agreed by most authorities. However, it is only mildly so. Its relation to other infectious diseases is little understood. While the disease is apparently decreasing in certain parts of the world (England), it still remains in other countries as a serious and increasing menace. Until its cause is definitely known it is impossible to formulate rules for its prevention. At present it is only possible to employ ordinary hygienic measures which are indicated for any infectious and communicable disease. No vaccine or serum is as yet available for its prevention or treatment.

ACUTE ENCEPHALITIS: AUSTRALIAN X DISEASE

In 1926 Kneebone and Cleland (42) reported an acute encephalitis condition which appeared again in Australia during January and February of the year 1925. In previous reports Cleland and Campbell (43) had reported an epidemic of acute encephalomyelitis which appeared in Australia in 1917–19. According to these authors the Australian disease, X disease, does not correspond with that of encephalitis lethargica reported in other parts of the world. Australian X disease is characterized by its high mortality, very high temperature, coma, convulsions, relative absence of eye symptoms, rapid approach to death in fatal cases, its general resemblance to cerebrospinal fever, and leucocytosis. Furthermore, these investigators have offered evidence that the virus of X disease may be transmitted to sheep.

During 1917 and 1918 the virus of X disease was transmitted successfully to monkeys and at the same time was apparently transmitted to a series of sheep. Successful "takes" were obtained into the third series of sheep, by sheep to sheep inoculation, but failed in the fourth. In 1926 Kneebone and Cleland again attempted to transmit the virus to these animals. Five sheep were inoculated. The first was inoculated directly into the brain substance with 1.5 cubic centimeters of nerve-tissue emulsion from a fatal human case of the disease, and died eight days later with general convulsions. The second died with similar symptoms nine days following inoculation. The third died with general convulsions seven days following inoculation. The fourth was inoculated with brain emulsion from sheep 3 and died with general convulsions nine days after inoculation. The fifth was inoculated with brain substance from sheep 4 and suffered no ill effects following the inoculation. Brains from sheep 1, 2, 3, and 4 all showed intense pial congestion but no evidence of suppuration. Bacteriologic cultures were uniformly negative.

The case described from which brain substance was obtained for the sheep inoculations is perhaps typical of the fatal cases of this disease. The authors described it as follows:

> *Case 3.* (From this case the sheep inoculations were made.) Lucy S., aged 2 years and 10 months, was admitted on March 1, 1925. She had had whooping cough ten months before, but since then had been quite well up to two days ago. For the last two days she has had "turns" each day, lasting up to six minutes at a time, and three convulsions, followed by general twitchings during the day before admission. The bowels had been opened once daily. She had refused food since she became ill. Examination showed that she was in a semi-comatose condition, with generalized twitchings. Head retraction and Kernig's sign were present. Nothing abnormal was detected in the chest or abdomen. The urine showed no abnormality. The temperature was 101.6° F., the pulse 140, and respirations 48. Next day her condition was worse, although the twitchings were not so severe. There was left abducent paralysis. Lumbar puncture yielded 60 cc. of clear fluid under slightly increased pressure. This fluid contained no increase of globulin, no pus cells, and no organisms either in smears or on culture. The temperature, 101.6° F. on admission,

rose in 12 hours to 105° F., and maintained a high level, rising to 106° F. a few hours before death, which occurred 36 hours after admission. At the post-mortem examination, 12 hours after death, the brain was removed with strict aseptic precautions. Very marked congestion of the vessels of the pia mater was present, both at the vertex and at the base. There was no evidence of tuberculosis or suppurative meningitis, and the other organs were healthy. Microscopic examination of sections from the brain and spinal cord failed to reveal the presence of perivenous sheaths of cells or of cellular islands. These sections included the basal muclei. There was marked capillary congestion in the frontal and occipital areas.

From the description of this case there can be no doubt that the case is in many ways dissimilar to epidemic encephalitis both in its symptomatology and in its morbid anatomy. While the transmission of the virus to sheep is not conclusive, these experiments may be considered as strong evidence that such transmission is possible. If this point is accepted it is also important evidence that the disease is at variance with encephalitis lethargica for no virus has ever been transmitted to sheep from cases of epidemic encephalitis or from cases of poliomyelitis. Indeed, in our experience it has been impossible to infect sheep with herpes virus or, for that matter, satisfactorily to demonstrate antibodies in the blood stream of sheep that have received more than a dozen injections of herpes-virus emulsion in an attempt to specifically immunize them.

What the exact nature of Australian X disease is and its microbic incitant must be left for future experimental work to decide. For the present we must regard this disease as still another form of encephalitis, and most probably it is unrelated to encephalitis lethargica.

JAPANESE ENCEPHALITIS: TAKAKI VIRUS

In 1924 there was an epidemic of encephalitis in Japan. This epidemic occurred in the summer and involved about six thousand cases. Various investigators attempted to transmit the disease to animals. Takaki (44) has reported that he succeeded in six transmissions of the virus of the disease to rabbits with material from six fatal cases. According to this investigator the virus is inoculable by both cornea and brain as well as other organs. It cannot be cultivated artificially. Takaki states that the symptoms produced in rabbits by this virus are not similar to those induced in rabbits with the herpes virus. Furthermore, cross immunity tests with the Takaki virus and herpes virus show that there is no relation between the two viruses.

Commenting upon the Japanese disease in a recent paper Flexner(45) states:

> In view of this discordant finding, the question arises whether the Japanese and the European epidemic diseases are pathologically the same. Fortunately this question can be answered, and apparently in the affirmative. The clinical and pathological descriptions which have been published show close similarity. Through the kindness of Professor Kimura, of the Imperial University in Sendai, I have been enabled to examine specimens taken from the brain of fatal cases. These specimens show pathological changes closely resembling those found in the brain of Europeans and Americans who have succumbed to epidemic encephalitis. The changes or lesions are of two sorts: mononuclear (lymphoid) infiltrations of the blood vascular sheaths and brain tissue, and degeneration of ganglion and glia cells. The distribution of the lesions is also typical. Especial attention may be drawn to the lesions in the substantia nigra which are prominently present in the Japanese, as well as in the European cases of the disease.

This author calls attention to the virus reported by Kling and Liljenquist(46) in Sweden in 1921 which these investigators obtained from a case of epidemic encephalitis. The experimental disease produced by this virus produces a chronic pathological process rather than an acute process and in this respect differs from all the other viruses which have been reported. Some investigators take the stand that Kling and Liljenquist were dealing with the virus of spontaneous encephalitis in rabbits, but Flexner states:

> There is no doubt that the Swedish cases of epidemic encephalitis are identical with the other European and the American cases. . . . there is strong reason to believe that the Japanese epidemic disease is of the nature of the European and American disease. The essential differences relate to the microbic incitant described by Kling and by Takaki. As tested by these discrepancies, the epidemics would have to be regarded as distinct. The fundamental question raised by the discrepancies is, therefore, whether the experimental findings are not open to the suspicion of not revealing the real incitant of the epidemic disease.

For the present the Japanese epidemic of encephalitis must be regarded as related to epidemic encephalitis as it occurs in other parts of the world. Its virus is one of the few which has been isolated from the brain tissue of human cases of the disease and is unique in its unlikeness to the herpes virus.

VACCINATION ENCEPHALITIS

Within the last two or three years there have appeared cases of encephalitis which have apparently been related to vaccination. Turnbull and McIntosh,(47) Heymann,(48) Aldershoff,(49) and others have written reports upon this subject. Heymann quotes Jenner's observation on the inhibition of the development of the smallpox-vaccine pustule in patients with herpes. Turnbull and McIntosh have reviewed seven cases of encephalomyelitis which were definitely connected with vaccination. The only virus demonstrated experimentally in the brain and cord was a vaccinal virus. The postvaccinal encephalitis develops from nine to fifteen days after the vaccination.

Levaditi, Nicolau, and Bayarri(50) regard this form of encephalitis as the flaring up of a latent virus of epidemic encephalitis already present in the central nervous system. This theory is also concurred in by Netter.(51) Bastiaanse, Bijl, and Terburgh(52) support a similar idea. Fiedler(53) states that fifty-two instances of disease of the central nervous system after vaccination are recorded, and he believes that vaccination activates viruses already present in the system.

Lucksch(54) on the other hand maintains that encephalitis following vaccination is caused by the vaccine virus. Wilson and Ford(55) point out that a diffuse nonsuppurative encephalomyelitis may occur as a rare but specific complication of variola, vaccinia, and varicella. These authors further state that the vaccine virus has been demonstrated in the cases of encephalitis following vaccinia but not in connection with variola and varicella. These authors believe that the condition is in reality uncommon and that many such cases have been mistaken for tetanus. Encephalitis has also been reported in connection with measles, mumps, etc.

Lucksch has suggested the use of the Paul test with spinal fluid from these cases. He believes that corneal lesions may be obtained on rabbits with spinal fluid from these cases which may be regarded as characteristic of smallpox virus.

This question is of special interest from the medico-legal point of view, particularly if the possibility of tetanus infection is involved.

SPONTANEOUS ENCEPHALITIS IN RABBITS

The study of experimental encephalitis in rabbits has been complicated by the presence in these animals of spontaneous encephalitis. Spontaneous encephalitis in rabbits was first noted by Bull(56) in 1917. Bull was studying the brains of rabbits inoculated with streptococci and found focal areas of necrosis surrounded by lymphocytic cells. These areas were near the blood vessels showing a perivascular infiltration extending from the meninges. Subsequently Bull examined several hundred rabbits having snuffles and found one which had acute meningitis with perivascular infiltrations within the cerebrum. Reasoner(57) in 1916 had noted similar changes in animals that had received intravenous injections of syphilitic material. These observations indicated that the stock animals might be originally affected by this process and that it bore no relation to the experimental procedures being studied.

In 1922 Oliver(58) first spoke of a spontaneous chronic meningo-encephalitis of the rabbit. This author found about 20 per cent of stock animals and animals bought on the market showing the changes noted above. The animals were in apparently healthy condition. During the same year Twort(59) described spontaneous encephalitis in a litter of rabbits that was characterized by definite symptoms. The onset was insidious, the temperature subnormal, the hair fell out, the animals lost weight, and there was a discharge from the eyes. Later muscular weakness progressed, the animal developed convulsions, and died in coma. Twort and Archer later transmitted the malady to four young rabbits and attempted to link this virus with nephritis in animals.

McCartney(60) found that about half of three hundred seventy-two rabbits examined had such lesions and that in certain groups of animals as high as 76 per cent were affected. The lesions consisted of perivascular, meningeal, parenchymatous, and subependymal infiltrations with mononuclear cells. Here and there were focal areas consisting of aggregations of cells. These areas were distributed throughout the brain. Necrotic foci were found in 15 per cent of the brains examined.

Later several investigators made a study of this condition in rabbits, among them Levaditi, Nicolau, and Schoen.(61) These authors noted a microörganism in the lesions which they designated *Encephalitozoon cuniculi.* The parasites were found next to the subcortical nodules of epithelioid and lymphocytoid cells and occasionally in the brain substance. They appeared to be in cysts, and usually twenty to forty microörganisms were found in each cyst. Similar organisms had already been described by Wright and Craighead in 1922 and by Doerr and Zdansky. Later, in 1924, similar organisms were described by Cowdry and Nicholson(62) who termed them protozoan-like parasites.

That spontaneous encephalitis exists in apparently healthy rabbits is an established fact. This knowledge should be carefully considered in connection with experimental studies with encephalitis material.

BIBLIOGRAPHY

1. Savage, Journ. Cut. Dis. **1** (1883) 253.
2. Seaton, Trans. Clin. Soc. London (1886) 26.
3. Grueter (1913). Cited by Lowenstein, Wien. klin. Woch. **32** (1919) 952.
   Deutsch. opth. Gesellschft. (Aug. 6, 1920).
   Deutsch. med. Woch. **48** (1922) 1156.
4. Lowenstein, Wien. klin. Woch. **32** (1919) 952; Münch. med. Woch. **61** (1919) 769; Wien. klin. Woch. **56** (1920) 1222. Klin. Monatsbl. f. Augenheilk **64** (1920) 15.
5. Luger and Lauda, Wien. klin. Woch. **34** (1921) 132 and 251; Zeitsch. f. d. gesammt. Exper. Med. **24** (1921) 289.
6. Blanc and Caminopetros, Comp. rend. Soc. Biol. **84** (1921) 629, 767, 859; **85** (1921) 290.
7. Levaditi, Harvier, and Nicolau, Ann. Inst. Pasteur **36** (1922) 63.
   Levaditi and Harvier, Ann. Inst. Pasteur **34** (1920) 911.
8. Lipschütz, Arch. f. Dermat. u. Syph. **126** (1921) 428.
9. McKinley and Holden, Arch. Path. and Lab. Med. **4** (1927) 155.
10. Goodpasture and Teague, Journ. Med. Res. **44** (1923) 121.
11. Howard, Am. Journ. Med. Sci. (1903) 256; (1905) 1012.
12. Mallory and Wright, Mass. State Board of Health Reports (1889).
13. Doerr and Vöchting, Rev. gén. d'opht. **34** (1920) 409.
14. Cole and Kuttner, Journ. Exp. Med. **42** (1925) 799.
15. McKinley and Holden, Journ. Infect. Dis. **39** (1926) 441.
16. Le Fèvre and McKinley, unpublished data (1924–25). Cited ibid.
17. Parker, and Nye, Am. Journ. Path. **1** (1925) 337.
18. Economo, Wien. klin. Woch. **30** (1917) 581.
19. Loewe and Strauss, Journ. Am. Med. Assoc. **73** (1919) 1056.
20. Flexner, Journ. Am. Med. Assoc. **81** (1923) 1688, 1785.
21. Doerr and Schnabel, Ztschr. f. Hyg. u. Infektionskrankh. **94** (1921) 29; Schweiz. med. Woch. **51** (1921) 469; **52** (1922) 325.
22. Doerr and Berger, Schweiz. med. Woch. **52** (1922) 863.
23. Zdansky, Frnkf. Ztschr. f. Path. **29** (1923) 207.
    Doerr and Zdansky, Schweiz. med. Woch. **53** (1923) 349, 1189; **54** (1924) 150; Ztschr. f. Hyg. u. Infektionkskrankh. **102** (1924) 1.
24. Schnabel, Klin. Wchnschr. **1** (1922) 1685; Wien. klin. Wchnschr. **36** (1923) 84.
25. Koritschoner, Wien. klin. Wchnschr. **2** (1923) 385.
26. Bessemans and van Boeckel, Comp. rend. Soc. de Biol. **88** (1923) 1225; **89** (1923) 109.
    Van Boeckel, Bessemans, and Nelis, L'Encéphalite léthargique. Nossent & Co., Brussels (1923).
27. Da Fano, Medical Science **10** (1924) 355.
28. Perdrau, Brit. Journ. Exp. Path. **6** (1925) 41.
29. Flexner and Amoss, Journ. Exp. Med. **41** (1925) 233, 215, 357.
30. Doerr, Zentr. Haut-u. Geschlechtskrankh. **13** (1924) 417; **15** (1924) 1, 289; **16** (1925) 481; Centr. Bakt., l. Abt., Orig., **97**. suppl. (1924–26) 76.

31. PARKER, Journn. Med. Res. 44 (1923–24) 289.
32. ZINSSER and TANG, Journ. Exp. Med. 44 (1926) 21.
33. FLEXNER, Journ. Exp. Med. 47 (1928) 23.
34. ROSENOW, Journ. Infect. Dis. 34 (1924) 329.
35. EVANS and FREEMAN, Pub. Health Rep. 41 (1926) 1095.
36. EVANS, Pub. Health Rep. 42 (1927) 171.
37. FREEMAN, Journ. Am. Med. Assoc. 89 (1927) 1317.
38. RUSSELL, Candad. Med. Assoc. Journ. 12 (1922) 705.
39. MACCALLUM, Medicine 5 (1926) 59.
40. BASTAI and BUSACCA, Wein. klin. Woch. 3 (1924) 147, 442.
41. MCCRAE, Osler's Principles and Practice of Medicine. Appleton & Co., New York and London (1926).
42. KNEEBONE and CLELAND, Australian Journ. Exp. Biol. and Med. Sci. 3 (1926) 119.
43. CLELAND and CAMPBELL, Rep. Director-General of Public Health, N. S. W. (1917) 150–280; Brit. Med. Journ. (May 31, 1919) 663; Med. Journ. of Aust. (March 22, 1919); Proc. Roy. Soc. Med. 13 (1920) 185; Journ. Hyg. 18 (1919) 272.
CLELAND, Proc. Roy. Soc. Med. 12, No. 9, Sec. Path. (1919) 33.
44. TAKAKI, Z. Immunitätsforsch. 47 (1925) 441; Japan Med. World 5 (1925) 147.
45. FLEXNER, Journ. Gen. Physiol. 8 (1927) 713.
46. KLING and LILJENQUIST, Comp. rend. Soc. Biol. 84 (1921) 521.
KLING, DAVIDE, and LILJENQUIST, Comp. rend. Soc. Biol. 84: 815; 85 (1921) 823; 87 (1922) 75.
47. TURNBULL and MCINTOSH, Brit. Journ. Exp. Path. 7 (1926) 181.
48. HEYMANN, Deutsche med. Wchnschr. 52 (1926) 442.
49. ALDERSHOFF, Nederl. Tijdschr. v. Geneesk. 70 (1926) 267.
50. LEVADITI, NICOLAU, and BAYARRI, Presse Médicale 35 (1927) 161.
51. NETTER, Quoted from Comby, Bull. Soc. Med. dés Hop. 50 (1926) 1434.
52. BASTIAANSE, BIJL, and TERBURGH, Nederl. Tijdschrift. b. Geneesk. 70 (1926) 1267.
53. FIEDLER, Ztschr. f. Kinderh. 42 (1926) 336.
54. LUCKSCH, Deutschr. Ztschr. f. d. ges. gericht. Med. 7 (1926) 203; Med. Klin. 20 (1924) 1170; 21 (1925) 1377.
55. WILSON and FORD, Johns Hopkins Hospital Bull. 40 (1927) 337.
56. BULL, Journ. Exp. Med. 25 (1917) 557.
57. REASONER, Journ. Am. Med. Assoc. 67 (1916) 1799.
58. OLIVER, Journ. Infect. Dis. 30 (1922) 91.
59. TWORT, Vet. Journ. 78 (1922) 194.
TWORT and ARCHER, Vet. Journ. 78 (1922) 367; Lancet 1 (1923) 1102.
60. MCCARTNEY, Brit. Med. Journ. 2 (1924) 1159.
61. LEVADITI, NICOLAU, and SCHOEN, Comp. rend. Soc. de Biol. 89 (1923) 984, 1157.
62. COWDRY and NICHOLSON, Journ. Am. Med. Assoc. 82 (1924) 545.

BORNA DISEASE

ENZOÖTIC MENINGO-ENCEPHALOMYELITIS OF HORSES

Definition.—Enzoötic meningo-encephalomyelitis of horses is an acute, specific, infectious disease affecting the central nervous system. The disease is characterized by multiple lymphocytic foci occurring in the nerve substance and in the meninges. In certain ganglion cells intranuclear formations have been described.

History.—It is doubtful if the disease known as "seatstroke" and described by Wörz in Würtemburg in 1813 was encephalitis. It is more probable that this disease was cerebrospinal meningitis, as was the disease that later, in 1824 to 1828, spread through Europe and was described by Franque as "fever of the nerves." During the period 1847 to 1869 a similar disease was prevalent in the northern American states and was described by Liautard.(1) Later, in 1879, the disease appeared in Saxony and was epizoötic from 1883 to 1886. From 1894 the disease spread with great virulence in some localities and was especially malignant around Borna. From that time it has been spoken of as "Borna disease." A similar condition has been described in Hungary and in Belgium, Marcq.(2) The earlier studies on the bacteriology and pathology of Borna disease were made by Siedamgrotsky and Schlegel (1896),(3) Johne (1896),(4) Ostertag (1900),(5) Dexler (1900),(6) Streit (1902),(7) Oppenheim (1907),(8) and Christiana (1909).(9) According to the histologic work of Dexler and of Oppenheim the disease is an inflammatory one.

Distribution.—The disease has been present in Germany, Hungary, Belgium, Great Britain, and Russia. In North America it has been prevalent in New York, Pennsylvania, New Jersey, Minnesota, Kansas, Illinois, and parts of Ontario.

The virus of Borna disease.—Many bacterial forms have been described as the cause of Borna disease. Streptococci and diplococci have been found in the brains of animals having died of the disease and such bacterial forms have not been found in the brains of healthy animals. Ostertag has described a streptococcus, Borna-streptococci; Lohr(10) a diplococcus, Borna-diplococci; and Joest(11) a diplo-streptococcus, Borna-diplo-streptococci, in the brain, spinal fluid, and other tissues of the body in cases of Borna disease. Some of the diplococci are Gram negative and others Gram positive. Others are found partly within cells. Ostertag has described them in the blood, liver, and urine, but only in a few cases were they present in the brain. In cultures they grew in short chains and in bouillon produced dense clouding. They grew on both acid and alkaline media and in media which contain ammonia or saltpeter.

The Borna streptococcus is not pathogenic for laboratory animals. Horses, sheep, and goats have developed symptoms quite similar to Borna disease when injected subdurally in several instances, but the results are not uniform. According to Ostertag injections of this organism under the skin, into the nasal cavity, the eyes, the ears, and into the digestive tract produce no effect. Joest advanced the theory that Borna disease is due to a filterable virus because of the appearance of intranuclear inclusions in the ganglion cells. In view of this the causative agent is to be regarded as a parasite similar to the Chlamydozoa of Prowazek.

In recent years the theory that Borna disease may be due to a filterable virus has gained weight in the experimental work of Moussu,(12) Zwick and Seifried,(13) Beck and Frohböse,(14) and of Ernst and Hahn,(15) all of whom believe that the causative agent in this disease is a filterable virus. Transmission in series of a filterable agent obtained from cases of Borna disease, when injected intracerebrally, has been obtained in the horse, sheep, rabbit, guinea pig, rat, and fowl.

Moussu, as well as Miessner,(16) has shown that enzoötic encephalomyelitis of sheep is due to a virus which is analogous to the virus that affects horses. This has been confirmed in the work of Beck and Frohböse. Ernst and Hahn have isolated from cases of enzoötic encephalomyelitis of cattle and from malignant catarrhal fever a virus closely related to if not identical with that previously isolated from Borna disease in horses. The viruses under study at the present time are the Zwick virus, isolated from Borna disease in horses, and the Miessner virus which was isolated from a sheep. In a recent publication Nicolau and Galloway(17) have described their experiments with these viruses.

According to these authors the virus when centrifuged for five to fifteen minutes at 5,400 revolutions per minute is still found to be present in the supernatant fluid. The virus in destroyed when exposed to the action of ultra-violet light for five minutes, though the distance at which the rays are permitted to act is not stated. Nicolau and Galloway report that cross immunity experiments with the Zwick virus and the Miessner virus are positive. The Zwick strain, according to their experiments with rabbits, will protect against one thousand infective doses of the Miessner strain. The incubation period in rabbits varies with the individual susceptibility of the animal, the age and the weight being the sole factors. For rabbits weighing 1,500 grams and over, the incubation period with the Zwick virus ranged from twenty-eight days to thirty-six days. For the Miessner virus the period of incubation was thirty days based upon twelve rabbits, for guinea pigs nineteen to one hundred thirty days, for mice thirty-seven to one hundred twenty-five days. The virus is not infective for guinea pigs when injected into the epithelial pads of the metatarsal region as is the case with the virus of foot-and-mouth disease and of herpes. These authors found that passage of the virus of Borna disease through guinea pigs apparently enhances its virulence for rabbits since rabbits died following the injection of such a virus after nineteen to twenty-one days and the lesions were most intense. Monkeys (*Macacus rhesus*) were found by them to be susceptible to the virus, the first symptoms of the disease appearing after fifty-seven days, and sixteen days later the animal died showing definite post-mortem signs of the disease. The ferret was found to be refractory to the virus.

In rabbits that had died of Borna disease definite histologic changes were noted by the above authors. There is a mononuclear meningitis and a perivascular and parenchymatous infiltration. These infilterative cells are mononuclear in type with oxyphilic corpuscles in the large ganglion cells of the cornu ammonis. These correspond to those described by Joest and Degen (18) in 1909. Nicolau and Galloway further noted a ganglioradiculitis, peripheral neuritis, and they suggest the name "satellitism" for lesions occurring in the medulla oblongata, mid-brain, and spinal ganglia, which consist of neurons surrounded by neuroglial cells and infiltrating mononuclear cells. This satellitism, so they state, may go so far as to constitute true neuronophagia. The stages and forms of degenerative processes described by these authors are oxychromasia, chromatolysis, karyolysis, and vacuolization of the cytoplasm. Neuronophagia may even occur in the anterior horns of the spinal cord. In the paravertebral ganglia neuronophagia is more frequent than in the central nervous system proper and is comparable to that seen in poliomyelitis in monkeys. Mononuclear and perivascular infiltration was also noted in the nerve roots and descending peripheral nerves.

The corpuscles of Joest and Degen (intranuclear oxyphilic formations) were found in cells that presented a marked rarification of the karyoplasm. As in the case of Negri bodies these bodies were not found in degenerated cells. These bodies were also in evidence in the pyramidal cells of the cerebrum, medulla, anterior horns of the spinal cord, and in the nerve cells of the spinal ganglia. Cysts were also found in about 5 per cent of the cases in the large ganglion cells of the cornu ammonis. The histologic changes in experimental Borna disease in guinea pigs, rats, mice, and monkeys were practically identical with the description given above for the rabbit. Similar changes are noted in the natural disease as it occurs in horses.

A form of encephalitis is also found in human beings and has been described in other animals such as the sheep and the goat. At present there is no evidence suggesting any relationship between Borna disease and encephalitis in human beings, though several of the histologic changes appear to be similar and authorities are agreed that encephalitis in human beings is probably a filterable virus. (See previous section.)

Symptoms in Borna disease.—Noack(19) in 1908 observed symptoms of Borna disease in a horse nine days after the animal had been introduced into an infected district. The exact period of incubation in the natural infection is unknown. The incubation period in the experimental disease has already been mentioned. The disease may begin with digestive disturbance, loss of appetite, jaundice, colic, catarrh of the pharynx and respiratory tract, periods of excitement and dullness, and in some cases spasms in the muscles of the head. There may be squinting, grinding of teeth, unequal dilatation of the pupils, spasmodic elevation of the alæ of the nostrils and lips, spasms of the neck muscles in which case the head is drawn back, spasms affecting the extremities, and in some cases there is a marked opisthotonus. There is a hyperæsthesia and markedly increased reflex irritability. Symptoms of paralysis appear early. The animal is at first lame and shows weakness of the limbs. Paraplegia or hemiplegia may develop. The rise in temperature is not high, the highest reported being 41.6° C. Usually the temperature remains around 39° C. Respiration and heart rate may be moderately increased. Emaciation is noted only late in the disease and then in only a small percentage of cases.

In experimental infections with the Zwick or Miessner virus in rabbits, Nicolau and Galloway noted a loss in weight, even to one-half the original weight of the animal, a subnormal temperature preceding death, and a marked increase in the mononuclear lymphocytes.

Animals susceptible to the virus of Borna disease.—Horses, sheep, rabbits, rats, mice, guinea pigs, monkeys, and fowls may be said to be susceptible to this virus. Ernst and Hahn believe that deer may also become spontaneously affected. It is probable that in the future other species may also be found susceptible to the virus.

Immunity in Borna disease.—As has already been mentioned artificial immunity may be produced in rabbits with either the Zwick or the Miessner virus. Cross immunity was positive between these two viruses. There is no report as to how long such an immunity persists in experimental animals.

In the natural infection among horses observation has led to the opinion that there is little, if any, immunity afforded by an attack of the disease. Later attacks may occur in animals that have recovered from a previous attack of the disease. No serum or vaccine has been prepared which is of any value in immunizing animals against this disease.

Control measures for Borna disease.—Isolation and quarantine measures are indicated for the control of this disease. Special attention should be given water and food supplies and if necessary pastures should be changed.

BIBLIOGRAPHY

1. LIAUTARD, Rec. (1869) 361.
2. MARCQ, Ann. (1909) 11.
3. SIEDAMGROTSKY and SCHLEGEL, A. f. Tk. 22 (1896) 287.
4. JOHNE, D. Z. f. Tm. 22 (1896) 371; S. B. (1896) 57.
5. OSTERTAG, B. t. W. (1900) 433; Z. f. Infkrh. 2 (1907) 152.
6. DEXLER, Z. f. Tm. 4 (1900) 110.
7. STREIT, B. t. W. (1903) 577.
8. OPPENHEIM, Z. f. Infkrh. 2 (1907) 148.
9. CHRISTIANA, A. f. Tk. 35 (1909) 253 (lit.).
10. LOHR, Beitr. z. Bakt. d. Gehirn-Bückenmarksseuche d. Pferde, Diss. Dresden-Leipsig (1910) (lit.).
11. JOEST, Z. f. Infkr. der Haust. 10 (1911) 293; Hd. d. p. M. 2, Aufl. 6 (1912) 251.
12. MOUSSU. *Vide* "L'encephalite enzootique des animaux domestiques" (1924) Paris. Vigot freres.
13. ZWICK and SEIFRIED, Berl. tierärztl. Woch. 40 (1924) 465; (with Witte) Ztschr. f. Infkrh. der Haust. 30 (1926) 42.
14. BECK and FROHBÖSE, Arch. f. Wissensch. u. prakt. Tierhk. 54 (1926) 84.
15. ERNST and HAHN, Müchen. tierärztl. Woch. No. 6 (Feb. 1927).
16. MIESSNER, Deutsche tierärztl. Woch. 34 (1926) 637.
17. NICOLAU and GALLOWAY, Brit. Journ. Exp. Path. 8 (1927) 336–341.
18. JOEST and DEGEN, Z. f. Infkr. 6 (1909) 348; 10: 293; 9 (1911) 1.
19. NOACK, S. B. (1908) 41.

OTHER REFERENCES

1. ARNDT, Ztschr. f. Infektionsk. d. Haust. 29 (1926) 184.
2. HUTYRA and MAREK, Path. and Therap. of the Dis. of Domes. Animals, 3d Eng. ed. Alexander Eger, Chicago (1926) (lit.).

POLIOMYELITIS: INFANTILE PARALYSIS

ACUTE ANTERIOR POLIOMYELITIS

Definition.—Infantile paralysis is an acute, systemic, infectious disease, occurring both epidemically and sporadically, and is caused by a specific filterable virus. The virus of poliomyelitis attacks the nervous system and may affect every part of the cord, although it is prone to localize in the anterior horns of the gray matter. The posterior horns, however, may be seriously involved. In some instances the brain itself may be affected, the changes as a rule being more pronounced in the base of the brain. Symptoms of spastic paralysis indicate that the meninges are also involved in certain cases.

History.—Poliomyelitis has undoubtedly existed since ancient times. To Underwood is generally given the credit for the first description of this disease. We have been unable to read the book "A Treatise on the Diseases of Children," published by this author in 1784, but we are told by Vaughan who has studied his work that this author undoubtedly described infantile paralysis at that time under the title "Debility of the Lower Extremities." Until recent years poliomyelitis was not recognized as an infectious disease. The literature of the last century spoke of this disease as the Heine-Medin disease because Heine(1) in 1840 definitely established the disease as a clinical entity, and Medin(2) in 1890 was the first to study carefully an epidemic of the disease and describe its various clinical manifestations.

The first outbreak of infantile paralysis in the United States in 1894 was described by Caverly.(3) This epidemic occurred in the state of Vermont. The first reported epidemic of this disease had occurred in Sweden in 1881, the disease appeared the next year in Italy, and in 1886 small outbreaks were noted in Germany, France, and Norway. During the decade 1890 to 1900 there were minor outbreaks of the disease in Italy, France, Australia, England, and the United States. The epidemic at Rutland, Vermont, in 1894 consisted of 132 cases. Between 1900 and 1910 epidemics of the disease increased in proportion and severity. The disease was practically pandemic in Norway and Sweden from 1903 to 1907. From 1907 to 1910 large epidemics occurred in several of the Eastern and Midwestern States including New York, Massachusetts, Iowa, and Minnesota. At the same time the disease was endemic in various parts of Europe. Up to 1910 the United States had contributed over five thousand of about eight thousand cases which had been reported in various parts of the world.(4) Rosenau states that from 1910 to 1914 there were over eighteen thousand cases of this disease reported in the United States and during 1915–16 there were thirty-one thousand five hundred more. In 1916 alone this author states there were twenty-nine thousand cases and six thousand deaths resulted from this disease. New York City reported in this epidemic nearly nine thousand cases with over two thousand four hundred deaths.

Distribution.—Poliomyelitis is essentially a warm-weather disease, yet cases are exceedingly rare in tropical countries. It does occur in the Tropics, however, but never in epidemic proportions. The disease also occurs in cold countries, as evidenced by the epidemic in Iceland in 1924. The greatest number of cases are reported during the summer months. As a rule the disease is manifested only by sporadic cases during the winter months, although cold-weather epidemics have been reported (Sweden, 1911).

Epidemiologists state that infantile paralysis is usually more prevalent in sparsely settled communities than in the large cities. Its incidence was extremely high in New York City during 1916, but as a rule it is highest in rural communities. From available statistics males are slightly more susceptible than females and 95 per cent of cases are found in children under 10 years of age. In the New York City epidemic in 1916 the case mortality rate was 26.96 per cent.

The virus of poliomyelitis.—In 1905 Geirsvold(5) isolated certain bacteria, especially cocci, from spinal fluids and tissue specimens from cases of infantile paralysis. In 1909 Landsteiner and Popper(6) attempted to infect monkeys with emulsified spinal cord obtained from a child who died on the fourth day of an attack of infantile paralysis. Both monkeys were injected intraperitoneally, and one monkey died on the eighth day following the inoculation. The second monkey developed paralysis on the seventeenth day and was sacrificed for study on the nineteenth day. Two monkeys were inoculated with cord emulsion from the second monkey, but apparently neither was affected by the injections.

While Landsteiner and Popper were unable to transmit the disease from monkey to monkey they were able to study carefully the anatomical changes produced in the first two monkeys. The examination of the cords of these two animals showed the pia to be infiltrated with small, round, deeply-staining cells chiefly along the anterior median fissure. The substance of the cord exhibited areas of inflammation chiefly in the cervical area.

These areas consisted of perivascular infiltration and diffuse infiltration into the substance of the cord. Hæmorrhages were also noted in the gray matter. These lesions were found also in the medulla, pons, and brain stem though not so intense. The ganglion cells of the anterior horns also showed evidences of invasion and degeneration. The lesions in the second monkey were similar but more pronounced in the lumbar region. Hæmorrhages were absent and there were fewer infiltrating polymorphonuclear leucocytes in the cord substance.

About the same time Knoepfelmacher (7) reported the successful transmission of this disease to a monkey in Vienna. This animal was also injected intraperitoneally and developed symptoms of paralysis on the twelfth day. A second monkey injected with material from the first monkey remained unaffected, although the histologic findings in the first animal agreed with the description given by Landsteiner and Popper. During the same year Landsteiner and Levaditi, (8) Leiner and Wiesner, (9) and Römer (10) succeeded in transmitting the virus of poliomyelitis by the intracerebral route to monkeys. Landsteiner and Levaditi also demonstrated that the virus is filterable, a fact which, unknown to them at the time, had been learned by Flexner and Lewis in the United States. Leiner and Weisner also found that paralysis resulted following the injection of the virus into the intestine or stomach.

In 1909–10 Flexner and Lewis (11) demonstrated independently that the infectious agent in poliomyelitis is a filterable virus. These authors were able at will to infect monkeys with bacteria-free filtrates by both subcutaneous and intracerebral inoculations. They were also able to pass this virus from monkey to monkey by means of filtrates and in this way study the incubation period of the disease in this animal, the clinical forms of disease, and the anatomical changes produced by the virus. Under the dark-field microscope Flexner and Lewis observed innumerable bright, dancing points that when stained with Loeffler's flagella stain presented minute roundish or oval particles which were absent in filtrates prepared with normal rabbit spinal-cord emulsions.

Flexner and Lewis used for material the spinal cords from two cases of infantile paralysis in human beings. One patient had died on the fifth or sixth day of the disease, the second succumbed on the fourth day. The spinal cord obtained from the latter case contained wide-spread lesions affecting both the gray and the white matter. Sixteen hours after death the cord was emulsified and injected intracerebrally into monkeys. Symptoms of the disease began to appear in from six to forty-eight hours before paralysis developed. Symptoms of paralysis were found to develop in from four to thirty-three days with an average period of about nine days. Altogether these authors infected eighty-three monkeys in their first study. Flexner and Lewis found that the virus of poliomyelitis, in common with many other filterable viruses, resists the action of glycerin for at least seven days and also resists drying over caustic potash for the same period. They also demonstrated that the virus of poliomyelitis retains its virulence when kept constantly frozen at —2 to —4° C. for at least forty days. The virus is destroyed in the filterate when heated for half an hour at 45 to 50° C.

Of eighty-three monkeys (of several species) infected with the virus of poliomyelitis by Flexner and Lewis only six failed to develop paralysis. These authors believe that they have detected some evidence of immunity in monkeys which had been previously infected and at least partially recovered, and were then reinoculated. They state that "in no instance did the second inoculation produce a frank renewal of the disease or appear to retard the progress toward recovery." In other experiments a heated vaccine prepared of infected spinal cord was tried for preventive purposes but in each case failed to protect the animal when given simultaneously with the test dose of virus.

The virus of infantile paralysis remained to be cultivated. Flexner and Lewis noted the clouding of serum bouillon when a Berkefeld filtrate of the central nervous-system tissues of poliomyelitic monkeys was added to it; an observation that was confirmed by Levaditi,(12) but which proved to be due to protein precipitate and not to virus. In 1913 Proescher(13) found in stained smears from the nerve tissue of poliomyelitis-infected monkeys certain coccuslike bodies. Such bodies have also been described in rabies and their precise nature is unknown. They have not been found in poliomyelitis material taken from human cases.

In 1913 Flexner and Noguchi(14) described the cultivation of the virus of poliomyelitis. The medium consisted of human ascitic fluid to which was added a fragment of sterile fresh tissue. In the initial cultures the exclusion of oxygen is necessary, but it is sufficient to cover the liquid with paraffin oil for this purpose. Also cultures were obtained without fresh rabbit tissue by using fragments of poliomyelitic brain in ascitic fluid. Brain-tissue extract and sheep-serum water served nearly as well as ascitic fluid, especially when fresh rabbit tissue was added to the medium.

The virus of poliomyelitis stains by both the Giemsa and the Gram method, and in stained smears it appears as variable numbers of minute globoid bodies, arranged in pairs, or short chains, or in small aggregated masses. In the fluid culture the pairs and chains predominate. Once growth has been obtained in fluid medium, subcultures can be made to solid medium consisting of agar, ascitic fluid, and a fragment of sterile tissue. On solid medium the organisms develop as pairs and as aggregated groups. Cultures of the poliomyelitis virus were obtained by these authors from fresh poliomyelitic brains (monkeys), from filtrates prepared from infected brains, and from glycerinated poliomyelitic brains.

The individual organisms average about 0.2 micron in diameter. The limits of visible bodies are 0.15 to 0.3 micron. The minute globoid bodies usually appear in cultures by the sixth or seventh day. Growth begins slowly but increases rapidly later on and is usually complete by the eighth to the twelfth day. The cultures remain unchanged for several days in the incubator and are preserved for several weeks in the ice box.

The virus of poliomyelitis is sensitive to ordinary disinfectants, being destroyed by a 1 : 500 solution of potassium permanganate, 1 per cent menthol in oil, 0.5 per cent salol, 5 per cent boric acid, and 1 per cent hydrogen peroxide. The virus remains virulent in milk and water for about thirty days according to Levaditi and Pasti.(15)

Incubation period.—The incubation period is usually short, from one to three days, up to fourteen days, with many instances of seven days. Aycock and Eaton(16) have studied the disease in families, and judging from secondary cases which became infected they estimate the incubation period at ten to eighteen days with the average at fourteen days. The experimental disease in monkeys demonstrated in the hands of Flexner and Lewis an average incubation period of 9.82 days. This was calculated from the time of inoculation until the first symptoms of paralysis occurred. It is not thought that the incubation period in human beings varies much from that in monkeys.

Symptoms.—In monkeys there is no immediate effect following the inoculation. Nothing unusual is noticed until six to forty-eight hours preceding the onset of paralysis. The animals then may show certain prodromal manifestations. These consist of nervousness and excitability, tremor of the head, face, or limbs, shifting gaze, hairs somewhat erect, and the animals prefer to remain quiet. The paralysis develops suddenly. Flexner and Lewis say, "In general it may be stated that any of the larger groups of voluntary muscles may be first involved." In eighty-one animals inoculated by these authors forty monkeys developed paralysis in the left leg, twenty-one in the left arm, ten in all four limbs, and eight monkeys showed bulbar and cerebral symptoms. With the development of paralysis in a large group of muscles, other muscles may be found weak or paralyzed. The paralysis is associated with marked incoördination resulting in violent pseudoconvulsions; epileptiform convulsions with tonic and clonic muscle spasms; and finally, according to Flexner and Lewis, sudden death of the apoplectiform type. These authors state that death has occurred within thirty minutes of the first appearance of cerebral symptoms.

In the naturally occurring disease in human beings the disease comes on suddenly and is characterized by fever, irritability, and in many cases with nausea and vomiting. Paralysis usually appears within three or four days. In some cases the paralysis is the first symptom noted; abortive attacks without paralysis may occur in epidemics. During the acute stages of the disease the spinal fluid contains an increased number of cells, usually two hundred or more, and an increase in globulin. Paralysis usually remains stationary for a few weeks, improvement is gradual, but in some cases recovery takes place within a few months. Residual paralysis is usually permanent, but improvement may follow with proper treatment.

Animals susceptible to the virus.—Man is apparently the natural host for infantile paralysis. Monkeys of many different kinds are susceptible to the virus. Flexner and Lewis were unable to infect guinea pigs, rabbits, horses, calves, goats, pigs, sheep, rats, mice, dogs, or cats with this virus. Man and monkeys alone remain susceptible.

Immunity.—One attack of infantile paralysis confers a high degree of immunity. Second attacks have been reported but are rare. As mentioned above Flexner and Lewis found that monkeys which had recovered from an attack of the disease were immune. The serum of immune monkeys possesses virucidal properties for the virus in vitro. There is no racial immunity to poliomyelitis; the majority of cases have occurred in whites. Various preparations of infected nerve tissue have been tried for vaccination, such as desiccated cord, heated cord emulsions, and chemically treated emulsions. None of these has been sufficiently established to warrant its use in human beings. A serum in horses has been prepared but its preventive and treatment qualities have not been studied sufficiently to make any statement regarding its use.

Recently Shaw and Thelander(23) have reported the intramuscular use of convalescent serum in the treatment of poliomyelitis. These authors studied a series of eighty-one cases of the disease of which forty-three received convalescent serum intramuscularly during the active stage of the disease. They conclude that the use of convalescent poliomyelitis serum early in the course of the disease is of distinct value provided sufficiently large doses are used, and, if necessary, repeated doses of potent serum are administered. That the results as pointed out by Shaw and Thelander are in line with scientific investigations is brought out in a paper by Flexner and Stewart(24) who state that the employment of convalescent serum in the early treatment of human cases of poliomyelitis is based on decisive experiments in monkeys. These authors state:

> These experiments are divisible into two classes, in one of which proof was brought that the virus of poliomyelitis and convalescent serum mixed in vitro does not produce experimental poliomyelitis, and in the other of which it was shown that under controlled experimental conditions the intrameningeal injection of the convalescent serum, even as late as from eighteen to twenty-four hours after an intracerebral injection of the virus, sufficed to prevent an otherwise certain development of the paralytic experimental disease.

Flexner and Stewart make a plea that should poliomyelitis occur in individuals previously injected with convalescent serum it is to be hoped that the facts will be published for future guidance and information. As in the case of some of the other filterable virus diseases it begins to appear that convalescent serum in poliomyelitis may prove a means of successfully treating and preventing the spread of this tragic condition.

Transmission.—There are many theories regarding the transmission of this disease. There is evidence that the disease is spread from person to person and that healthy carriers of the virus may exist. Flexner and Amoss(17) have shown that the virus is present in the nasopharynx of both monkeys and human cases of infantile paralysis early in the course of the disease. Other investigators have also found the nasopharynx of monkeys infected following experimental inoculation (Osgood and Lucas). Rosenau, Sheppard, and Amoss(18) were unable to demonstrate the virus in the nasal or oral cavities in convalescent cases. Amoss and Taylor(19) were able to produce infection by placing the virus upon this membrane.

Other theories of transmission have been considered; such as, the insect-borne theory, milk-borne infection, air-borne theory, the animal-reservoir theory, transmission through wounds, etc. Rosenau and Brues(20) have demonstrated that the virus may be transmitted from monkey to monkey through the bite of the stable fly. This work was confirmed by Anderson and Frost.(21) Epidemics have been described as being due to infected milk supply (1925 and 1926). It is possible to induce infection by way of the gastrointestinal tract, but it is not thought that infection usually takes place in this way. Neustaedter and Thro(22) have been able to induce infection in monkeys with dust collected from sick rooms and suggest this mode of transmission in certain cases. There is at present no evidence of a possible animal reservoir for the virus of poliomyelitis, and the theory of infection through wounds possesses nothing in its favor. It seems best to regard poliomyelitis as a contact disease, though many of its features cannot be explained upon this basis. Future epidemics may yield more-definite information upon this point.

Pathology.—The anatomical changes found in monkeys have already been briefly described. In human beings the lesions are not only in the nervous tissues but also in the parenchymatous organs and lymphoid structures. In the nervous system the virus attacks the meninges particularly of the cord and medulla. There is a cellular inflammation of the pia most marked around the blood vessels. The walls of the vessels are also infiltrated and their lumen narrowed. The vessels entering the nerve tissue are also affected. Anæmia, œdema, and hæmorrhages result. The secondary degenerative changes in the nerve cells of the pons, medulla, cerebrum, and cord are secondary to the lesions in the vessels. Transient paralysis may be due to œdema or temporary vascular obstruction from pressure. Permanent paralysis is due to degeneration and actual destruction of the ganglion cells. Any part of the central nervous system may be involved. The symptoms depend upon the extent of this involment. The changes are chiefly in the gray matter of the anterior horns and consist of acute degeneration. The nerve cells may disappear entirely, being replaced by leucocytes. Round-cell infiltration may be noted in the posterior horns, the columns of

Clarke, and the white matter of the cord. Similar changes are also noted in the spinal ganglia. Also in some cases similar lesions may be found in the pons, medulla, cerebellum, and cerebrum. In other organs lesions such as bronchopneumonia and acute parenchymatous degeneration of the liver and kidneys may be found. The thymus, Peyer's patches, and the mesenteric lymph glands may be swollen. At autopsy gross changes are found in the spinal cord, ganglia, and muscles. The affected part of the cord may be smaller than normal, the anterior nerve roots are degenerated, the affected muscles atrophied. The affected limb may be shorter and the bones may be smaller than those on the normal side.

Prevention.—Isolation of cases of this disease is indicated. All discharges should be properly disinfected. All articles with which the patient has come in contact should be sterilized. Visitors should not be permitted. Prophylactic Pasteurization of milk is indicated. In general common-sense methods of prevention apply. No definite program has been formulated by epidemiologists for the prevention of this disease, and probably none will be until more exact knowledge of its spread and transmission is available.

BIBLIOGRAPHY

1. HEINE, Beobachtungen über Lähmungszustände der unteren Extremitäten und deren Behandlung. Stuttgart, F. H. Kohler (1840).
2. MEDIN, Verhandl. d. x. internat. med. Cong., Berlin, 1891, 2, 6 Abth. (1890) 37–47.
3. CAVERLY, Journ. Am. Med. Assoc. 36 (1896) 1.
4. ROSENAU, Preventive Medicine and Hygiene. D. Applenton & Company, New York and London (1927).
5. GEIRSVOLD, Norsk Magazin f. Laegevid 3 (1905) 1280.
6. LANDSTEINER and POPPER, Zeit. f. Immunitätsforsch. Orig. 2 (1909) 377.
7. KNOEPFELMACHER, Med. Klin. 5 (1909) 1671.
8. LANDSTEINER and LEVADITI, C. R. de la Soc. de Biol. 67 (1909) 592; 67 (1910) 787.
9. LEINER and WEISNER, Wien. klin. Woch. 22 (1909) 1698; 23 (1910) 91.
10. RÖMER, Münchener med. Woch. 61 (1909) 2505.
11. FLEXNER and LEWIS, Journ. Am. Med. Assoc. 53 (1909) 1639; Journ. Exp. Med. 12 (1910) 227; Journ. Am. Med. Assoc. 54 (1910) 45.
12. LEVADITI, Presse méd. 18 (1910) 44.
13. PROESCHER, New York Med. Journ. 97 (1913) 741.
14. FLEXNER and NOGUCHI, Journ. Am. Med. Assoc. 60 (1913) 362; Journ. Exp. Med. 18 (1913) 461.
15. LEVADITI and PASTI, Ann. de l'Inst. Pasteur 25 (1911) 805.
16. AYCOCK and EATON, Am. Journ. Hyg. 5 (1925) 724.

17. Flexner and Amoss, Journ. Exp. Med. **29** (1919) 379.
18. Rosenau, Sheppard, and Amoss, Boston M. & S. J. **164** (1911) 743.
19. Amoss and Taylor, Journ. Exp. Med. **25** (1917) 507.
20. Rosenau and Brues, Month. Bull. Bd. Health Mass. **7** (1912) 314.
21. Anderson and Frost, U. S. Pub. Health Rep. **28** (1912) 1733.
22. Neustaedter and Thro, New York Med. Journ. **94** (1911) 13.
23. Shaw and Thelander, Journ. Am. Med. Assoc. **90** (1928) 1923.
24. Flexner and Stewart, Journ. Am. Med. Assoc. **91** (1928) 383.

DISTEMPER

DOG ILL; PASTEURELLOSIS CANUM, MALADIE DES CHIENS, MALADIE DU JEUNE AGE (FRENCH); STAUPE DER HUNDE (GERMAN); CIMURRO, MOCCIO CANINO (ITALIAN)

Definition.—Distemper is an acute, contagious disease of young carnivorous animals. It is characterized by fever, acute catarrh of the mucous membranes, often followed by a catarrhal pneumonia and in some cases by involvement of the nervous system.

History.—Distemper is said to have been introduced into Europe from Asia or Peru in the eighteenth century. Since that time this disease has appeared in all parts of the world but is still present chiefly in European countries. In 1905 Carré(1) established its etiology as a filterable virus, but Ferry(2) and McGowan(3) later described a bacterium as the cause of the disease and the true etiological agent has not been definitely decided. There is a general feeling on the part of investigators, however, that the causative agent is a filterable virus.

Distribution of distemper.—The distribution of this disease is now quite general all over the world. The disease has been reported in practically every part of Europe, in North America, South America, Asia, Malay States, and the Philippines.

Incubation period.—The incubation period in artificial infection ranges from three to four days. It is somewhat longer in natural infection and is said to be as long as two weeks in some cases.

Symptoms.—In the paracute form of the disease the symptoms come on suddenly. There is marked depression accompanied by high fever, and the animal becomes quickly exhausted. After two or three days the temperature becomes subnormal, the animal becomes comatose, and death soon follows.

In the acute form the temperature rises to 40° C. or more and remains fairly high for one or even two weeks. It may, however, fall within a few days. If pneumonia develops the temperature goes up again and falls to subnormal just before death. The animal at first becomes depressed, will not play or obey calls, and its appetite is impaired or lost altogether. The hair is roughened, and sudden noises startle the animal and he trembles all over. An acute catarrh of the respiratory passages develops, and the animal develops an itching sensation on the nostrils. The discharge, which is first serous but later purulent, is very irritating and may be streaked with blood. A cough develops which indicates involvement of the larynx and bronchi. Vomiting may follow, and respiration becomes difficult and very rapid due to the short excursions of the diaphragm. As pneumonia develops catarrhal murmurs are heard over the chest wall. The eyes are frequently involved in the catarrhal process. They become swollen and finally shut. Keratitis and iritis may follow, the cornea becomes congested and the pupils become constricted.

The intestinal catarrh develops early and may at first give rise to constipation and later to diarrhœa. The stools may be streaked with blood and mucus. The urine may contain albumin and casts.

The nervous system is usually involved, and these symptoms may dominate the entire picture of the disease. Though the first symptom may be depression, the aminal has periods of marked excitement. Local and general muscular tremors and clonic spasm develop at times simulating epileptiform fits. These attacks exhaust the animal and usually lead to coma followed by death. The convulsions may become less frequent and finally disappear, but the animal is left with various degrees of paralysis of the extremities or local areas such as one side of the face, etc.

In about half of the cases a pustular exanthema develops on the skin. These lesions may involve particularly the orifices of the body and due to irritation may develop into ulcers that are slow in healing. A generalized septic condition may follow the appearances of ulcerative lesions. As death approaches the animal becomes very emaciated; it passes into coma and dies in convulsions.

Animals susceptible to the virus of distemper.—Dogs, particularly young dogs, are chiefly affected with the virus of distemper. Older dogs are not very susceptible, and this is thought to be due to the fact that they have experienced a form of the disease when they were younger. The susceptibility of cats is not high. Horses are said to be affected with distemper, but the relation of the virus of distemper in dogs and the virus of distemper in horses is not altogether clear. For the most part this disease may be considered a disease of dogs, and in all languages it is spoken of as dog sickness.

The virus of distemper.—The opinion of Carré that distemper is caused by a filterable virus has already been alluded to. Carré's experiments have been confirmed by Lignieres.(4) According to Carré the virus is present in the nasal discharge and in the blood early in the course of the disease. If such material is filtered and injected into a susceptible animal typical distemper is produced. Injected animals are found to have their pericardial sacs filled with a yellow serous fluid; and this fluid, if filtered and injected into other susceptible animals, is capable of producing the disease. Sinigaglia(5) and Babes and Starcovici(6) have demonstrated small bodies, that measure from 1 to 9 microns in size and closely resemble Negri bodies, in the epithelial cells of the respiratory tract, in the conjunctiva, in the Purkinje cells, in the nerve cells of the spinal cord, and in the ependyma cells of the walls of the ventricles. These authors believe these bodies to be parasites which develop from the virus granules. Standfuss(7) and Lentz(8) have also described bodies that they designate "distemper bodies," which are found in the nerve cells and outside of them. Other authors regard these bodies as degenerated nerve cells.

Ferry states that the nasal mucus of dogs affected with distemper contains a small, Gram-negative bacillus, and that this organism is found in the blood of affected animals in about 12 per cent of cases. The introduction of this organism into the nasal cavity resulted in a disease closely simulating distemper. Animals naturally resistant to distemper were also resistant to this organism, and animals infected with this organism were later found to be immune to the natural disease when exposed to it in other animals. Serum from naturally infected dogs agglutinated this bacterium in a dilution as high as 1: 800, the same as the serum from artificially infected dogs.

While this is strong evidence for the bacterial nature of this disease, it must be remembered that normal dogs harbor many kinds of bacteria which are not concerned in any disease process. Jensen(9) has found streptococci, staphylococci, and a bacillus similar to the influenza bacillus in normal dogs. Piorkowski(10) has described a slender capsulated bacillus in the tissues of healthy dogs, and Lignieres has demonstrated a small bipolar ovoid bacillus as a normal inhabitant.

Since filtrates from the nasal secretion are capable of producing the disease in susceptible animals opinion must lean to the filterable virus nature of the disease. According to this concept the bacterial form that has been described must be considered as a secondary invader.

The infection occurs through direct and indirect contact with infected animals. Infection may occur through the ingestion of food and water contaminated with the virus, and in addition there is evidence that it may occur by way of the respiratory tract. (Kelser.(11))

The virus of canine distempter is very susceptible to antiseptics and is destroyed within twenty minutes after exposure to 58° C.

Immunity in canine distemper.—One attack of the disease confers a lasting immunity. Younger dogs are more susceptible to infection with the virus of distemper than are older dogs. All attempts to produce a vaccine for preventive measures or a serum for curative use have failed. The best these products can hope to offer at present is to increase the normal resistance of the animal by stimulating in a small measure the normal resisting powers in the sense of nonspecific protein therapy.

Pathology.—The most notable anatomical changes in distemper occur in the respiratory organs. The mucous membranes are covered with a seropurulent exudate and small purulent plugs may be expressed from the lung tissue. In most cases there is a catarrhal pneumonia. The pericardial sac is filled with a yellow serous fluid. The pleura may be covered with a fibrinous exudate. There is acute catarrh of the mucous lining of the gastrointestinal tract and ulceration may take place. The liver and kidneys show parenchymatous degeneration. The eyes in addition to the catarrhal condition may show keratitis and in rare cases a panophthalmitis. Dexler(12) describes the changes in the nervous system as consisting of a marked myelitis and poliomyelitis. The inflammatory foci are present everywhere in the nervous system.

Control measures.—Common sense in isolating infected animals and preventing the transportation and importation of infected animals is indicated. There is no satisfactory, established method of inducing artificial immunity.

Distemper in cats.—While young cats are susceptible to the virus of distemper, the disease is less common in cats than in dogs. In cats the nervous symptoms are not so marked as in dogs, and the exanthema is said not to occur in the cat.

BIBLIOGRAPHY

1. CARRÉ, Bull. de la Sociéte centr. de méd. vet. (Paris) (1905) 335; Rev. Gen. **7** (1906) 649; C. R. (1906) 962.
2. FERRY, V. J. (1912) 376 (lit.).
3. MCGOWAN, Journ. Path. and Bact. **15** (1910).
4. LIGNIERES, Bull. (1900) 469 (lit.); Bull. (1906) 622; Physalix Bull. (1901) 131.
5. SINIGAGLIA, Atti del Congresso, Turin (1912) 256; Clinical Vet. (1912) 421.
6. BABES and STARCOVICI, C. R. Soc. de Biol. **73** (1912) 229.
7. STANDFUSS, Arch. f. Thierheilkunde **34** (1908) 109.
8. LENTZ, Zeit. f. Hyg. u. Infekt. **62** (1909) 63.
9. JENSEN, Maanedsskr (1895–96).
10. PIORKOWSKI, B. t. W. (1905) 830; (1906) 377.
11. KELSER, Manual of Veterinary Bacteriology. Williams and Wilkins Co., Baltimore (1927).
12. DEXLER, Arb. d. Wiener Inst. f. Nervenanat (1892); D. t. W. (1909) 313 (lit.).

OTHER REFERENCES

GOLDBERG and VOLGENAU, Cornell Vev. **15** (1925) 181.

KANTOROWICZ and LEWY, Arch. f. wissensch. u. prakt. Tierheilk **49** (1922–23) 137.

ROMAN and LAPP, Journ. Am. Vet. Med. Assoc. **66** (1924–25) 612.

SANFELICE, Centralbl. f. Bakt. **76** (1915) 495.

JESS, Zbl. f. Bakt. **25** (1899) 541.

WIRTH, T. Zbl. (1908) 200.

RICHTER, Die Hundestaupe, Diss., Bern (1908).

KREGENOW, Zbl. f. Bakt., L (1909) 326 (lit.).

LAMCHE, Diss., Zurich (1909).

ILLUSTRATIONS

PLATE 9

FIG. 1. Characteristic, peculiar, watchful and disturbed expression of the face in rabies.

2. Cervical ganglion of a dog affected with rabies. The nerve cells are partly atrophied, and in their place clumps of round cells appear. (After Van Gehuchten and Nélis; from Hutyra and Marek.)

PLATE 10

FIG. 1. Ammon's horn of a dog dead of rabies; *1*, Negri bodies in ganglion cells; *2*, a free Negri body; *3*, fragments of red blood corpuscles; *4*, artery. (After Hutyra and Marek.)

2. Infectious bulbar paralysis. Severe itching at the point of inoculation following subcutaneous infection. (After Schmiedhoffer; from Hutyra and Marek.)

PLATE 11

FIG. 1. Herpes simplex facialis. (From Hartzell.)

2. Herpes simplex. (From Hartzell.)

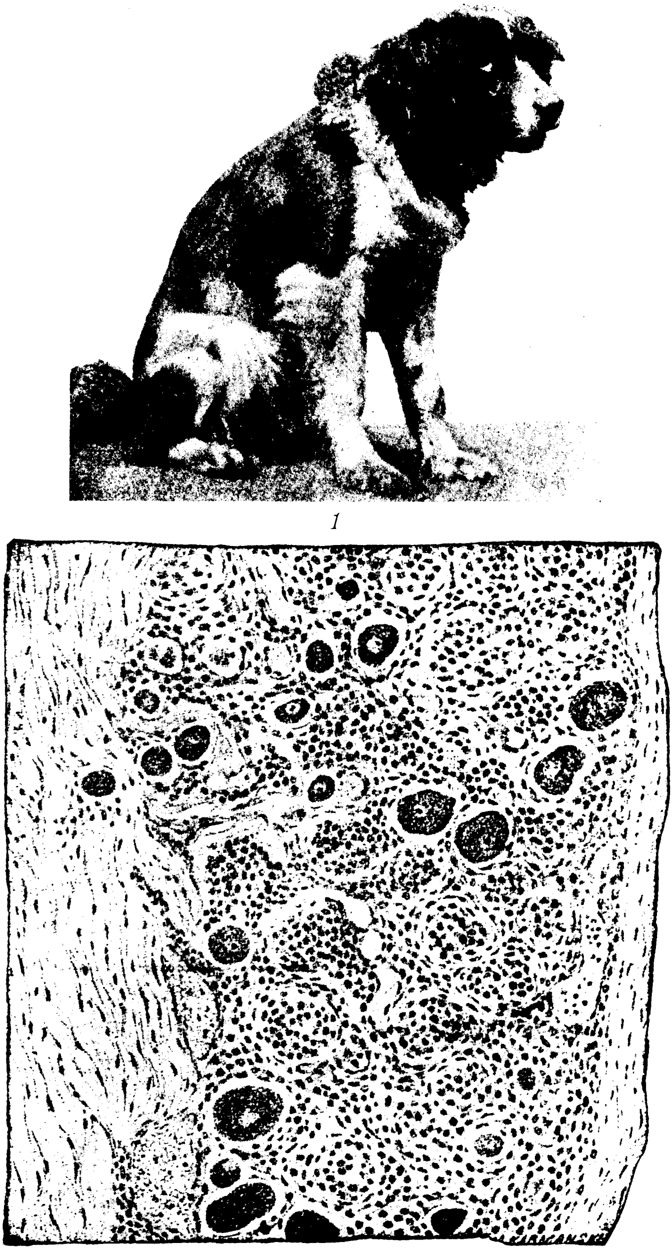

Fig. 1. Characteristic, peculiar, watchful and disturbed expression of the face. 2. Cervical ganglion of a dog affected with rabies.

PLATE 9.

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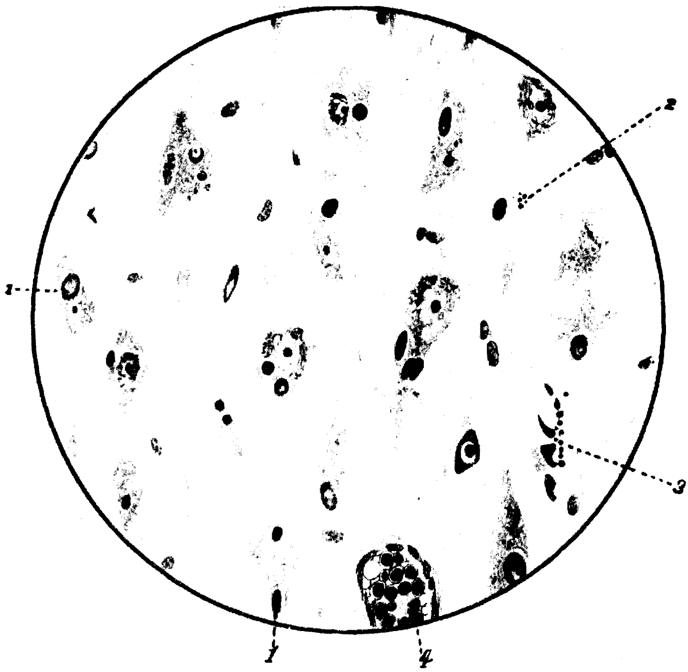

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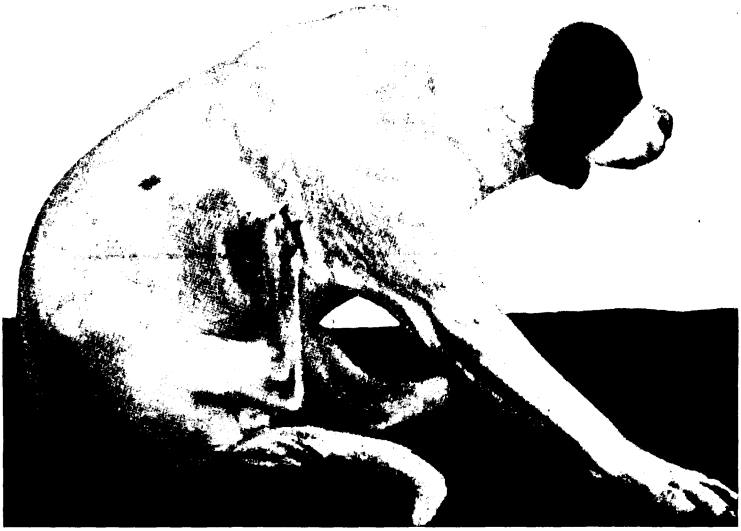

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Fig. 1. Ammon's horn of a dog dead of rabies. **2.** Infectious bulbar paralysis.

PLATE 10.

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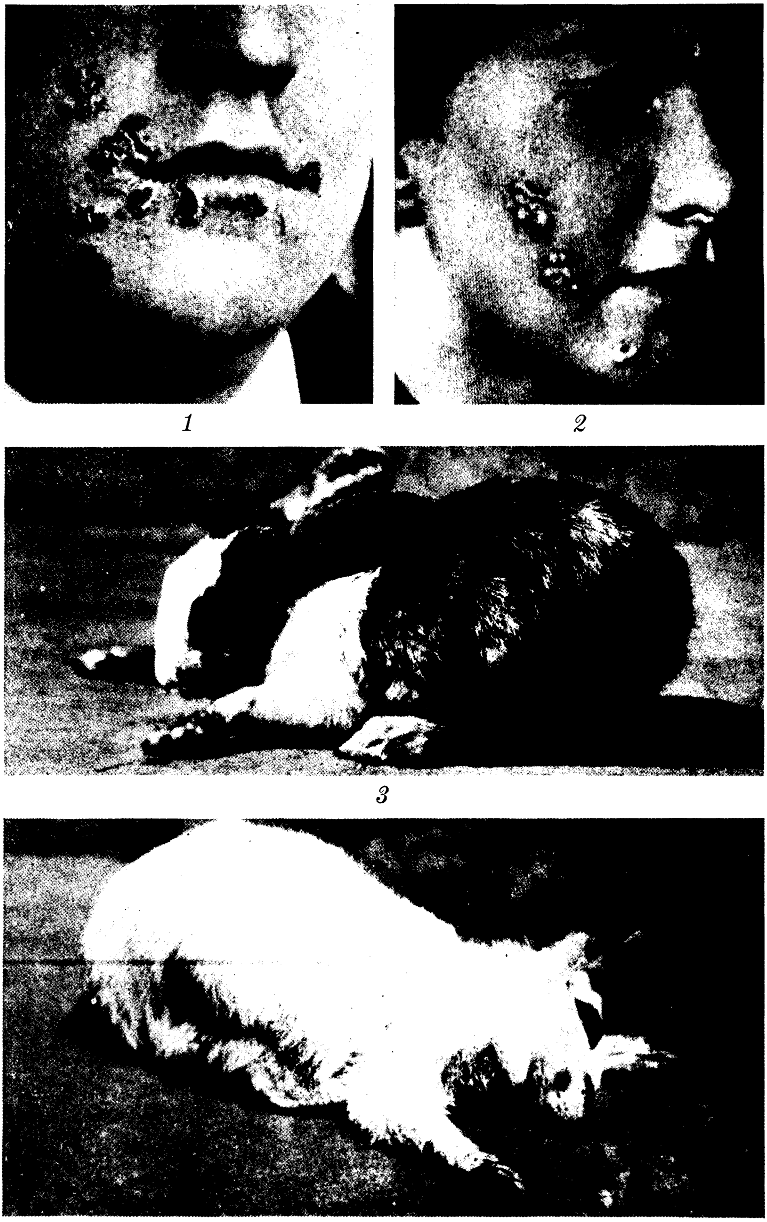

Fig. 1. Herpes simplex facialis. 2. Herpes simplex. 3. Experimental herpes encephalitis 4. Experimental encephalitis with herpes virus (Beckley strain).

PLATE 11.

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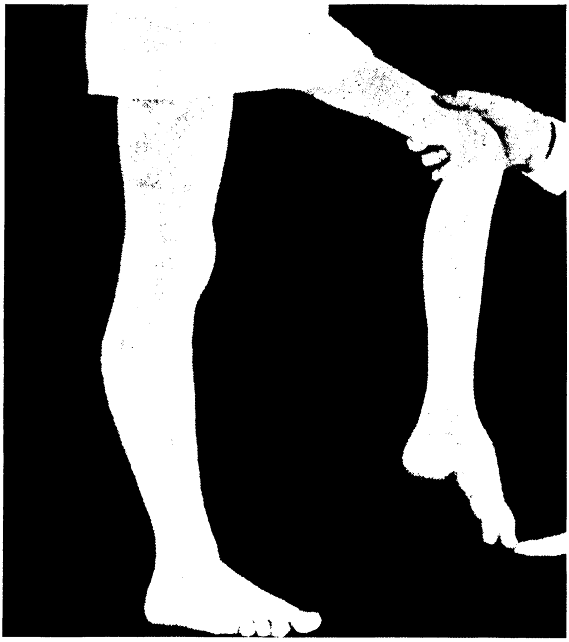

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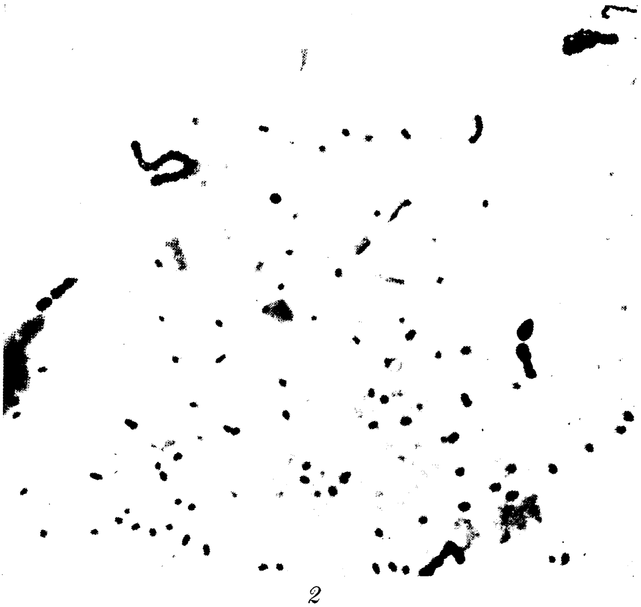

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Fig. 1. Old case of infantile spinal paralysis. 2. Microörganism causing epidemic poliomyelitis.

PLATE 12.

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FIG. 3. Experimental herpes encephalitis in a rabbit, the fourth day following infection.
4. Experimental encephalitis in a rabbit with herpes virus (Beckley strain).

PLATE 12

FIG. 1. Old case of infantile spinal paralysis, showing atrophy of the thigh and leg, and characteristic deformity of the foot. (After Holt.)
2. Microörganism causing epidemic poliomyelitis. (After Flexner and Noguchi.)

CHAPTER V

BACTERIAL DISEASES OF MAN AND ANIMALS (FILTERABLE)

SCARLET FEVER: SCARLATINA

Definition.—Scarlet fever is an acute, contagious, self-limited disease. It is characterized by fever, sore throat, and an erythematous rash appearing first upon the neck and spreading rapidly over the entire body. For many years this disease has been classified with the group of filterable virus diseases, although it has long been known that a streptococcus was associated with it. Adequate proof of the etiological significance of the streptococcus in scarlet fever was lacking, however, until the work of Dick and Dick(1) in 1921 to 1924, which has apparently settled the etiology of the disease. For the time being scarlet fever remains classified with the filterable group of diseases as one of bacterial origin.

History and distribution.—Vaughan(2) has published a very illuminating treatise on the history of this disease, and according to this writer the first definite records of scarlet fever were published in 1560. Vaughan states—

> An attempt has been made, notably by Malfatti, to ascribe the terrible epidemic, so vividly depicted by Thucydides, that swept Athens about 430 B. C. to scarlet fever, but we can find no reason for such a claim. Others have sought evidences of scarlet fever in the writings of Hippocrates, but the results of these researches have not been satisfactory. There is certainly no description given by the father of medicine of a disease which can be identified as scarlet fever. * * * The first description of the disease sufficiently definite to distinguish between it and measles was written by Ingrassias, of Palermo, (1560). In 1574 Baillou, under the name of rubiolae, described an epidemic in Paris and differentiated it from measles. A similar description by Coythar, of Poitiers, appeared in 1578. * * *
> We are indebted to Sydenham, who, in 1665 named and described this disease, thus giving it a definite and permanent place in the list of specific diseases.

In 1735 there was an epidemic of scarlet fever in New England which was spoken of as "throat distemper." In the following year Douglas described this disease as it existed in Boston. In 1789, according to Vaughan, Bard described scarlet fever as it occurred in New York, but Vaughan believes that the disease observed by Bard must have been laryngeal diphtheria. Since the eighteenth century epidemics of scarlet fever have been known in the United States and Europe, and to-day the disease exists in practically all temperate climates. For some unexplained reason scarlet fever is comparatively rare in tropical countries. Though it has been introduced into tropical countries, it does not spread. It is rare in India and South America, and in the Philippines it is seen chiefly in newly arrived Americans or other foreigners when it is seen at all. Other exanthematous diseases are not so rare, and the explanation for the comparative absence of scarlet fever remains obscure. Scarlet fever is a cold-weather disease. No explanation has been offered as to why scarlet fever should not appear in tropical countries or why the natives of these countries should possess an apparent immunity. It has occurred to us that metabolism in tropical countries might possibly be the reason for this. It is well known that fatty acids possess a high neutralizing power far bacterial toxins such as the hæmolytic streptococcus toxin of scarlet fever and the toxin of the diphtheria bacillus. In tropical countries little carbon is needed to maintain body heat and fats must be stored or utilized for other purposes. The suggestion is that through some metabolic process that differs from the usual metabolism of fats in temperate climates fatty acids become available for other purposes; for example, the neutralization of bacterial toxins. It is known, however, that other exanthematous diseases, such as measles, smallpox, and varicella, are not uncommon, and one might ask why the explanation offered for scarlet fever and diphtheria does not apply to these other conditions. In any event experimental proof of such a hypothesis is necessary before it is to be considered seriously. Whether or not melanin (containing sulphur) pigment possesses any neutralizing properties for bacterial toxins is not known. Possibly in colored skins this may be a factor. Streptococcus infections in general are less frequent in tropical countries than in temperate zones, which suggests that the family of streptococci does not find in these countries favorable conditions for growth and maintenance of virulence. These questions should receive more consideration from the standpoint of research in the future than they have in the past, and a solution of the problem may give new light on the more fundamental concepts of infection and immunity.

The virus of scarlet fever.—In 1885, Klein(3) described a streptococcus, which he isolated from the throat of a scarlet-fever patient, and designated it *Streptococcus scarlatinæ.* While the group of microörganisms designated as streptococci was known at this time, suitable methods in technic that would permit the grouping of these organisms into different strains had not been developed. It is only within recent years that the streptococci have been fairly accurately classified. It will be recalled that Pasteur (1879) had already pointed out the etiological significance of certain streptococci in puerperal sepsis, and organisms of this type had been described for other conditions; such as, erysipelas, septicæmia, lymphangitis, and abscesses. Also streptococci were found to be present in the throats of patients having measles, smallpox, influenza, pneumonia, and sinusitis. As a consequence of its general distribution the streptococcus etiology of Klein for scarlet fever could not stand, and the streptococcus was regarded by contemporary authorities as a secondary invader and probably without etiologic significance.

Further, it was known that scarlet fever produced an immunity against subsequent attacks, while other streptococcus infections produced little or no immunity. For this reason and the fact that the scarlet-fever streptococcus could not be differentiated from other streptococci, the strepotcoccus etiology of scarlet fever was discredited.

Baginsky and Sommerfeld(4) in 1900 demonstrated streptococci in the throats of seven hundred cases of scarlet fever. Moser(5) in 1902 prepared a polyvalent antitoxic serum with streptococci injected into horses, and this serum apparently gave some very promising results when tested on early cases of scarlet fever. Similar results were obtained in the hands of Schick.(6) That filtered broth cultures of streptococci obtanied from scarlet-fever cases contained a toxin was demonstrated by Savchenko(7) in 1905. This work was followed by that of Gabritschewsky(8) in 1907, who attempted to prepare a vaccine from broth cultures of streptococci. This vaccine had some prophylactic value, but the method of immunization never came into general use. This investigator found that about 15 per cent of children who received subcutaneous injections of small amounts of culture fluid developed a rash, sore throat, and strawberry tongue. When his material was administered to children who had recovered from an attack of scarlet fever no rash was produced. Undoubtedly this investigator was working with the toxin of the scarlet-fever streptococcus.

In 1918 Schultz and Charlton(9) discovered the interesting fact that convalescent scarlet-fever serum when injected into the skin of scarlet-fever cases produces a blanching. This has since been known as the Schultz-Charlton reaction. More recently Blake(10) has shown that of one hundred thirty-two cases of scarlet fever ninety-seven, or 72.5 per cent, gave this reaction, and this percentage would probably have been higher had every case been tested early in the course of the disease, and had the specific serums not been too highly diluted in some instances. It now appears to be well established that this reaction is specific for scarlet fever.

Dochez and Bliss(11) in 1920 studied the agglutination properties of streptococci and found that the hæmolytic streptococci of scarlet fever belong to a distinct group. Similar results were obtained by Tunnicliff.(12) In 1924 Dochez(13) produced an antitoxin for scarlet fever by injecting streptococci into a mass of agar-agar previously injected into horses. This antitoxin possesses definite therapeutic properties. Zinsser modified this technic by injecting into horses freshly infected blood before clotting had taken place.

From 1921 to 1924 there was a series of papers by Dick and Dick, of Chicago, who reported the experimental production of scarlet fever with pure cultures of hæmolytic streptococci in volunteers. These investigators found that not all hæmolytic streptococci associated with scarlet fever are of the same type and that the streptococcus found in the throat of scarlet-fever patients is rarely present in the blood stream. The toxin produced by their streptococci was found in filtered broth cultures of the virus, and typical symptoms of scarlet fever followed the introduction of this toxin into susceptible volunteers. The toxin is heat resistant and is neutralized by convalescent scarlet-fever serum. Susceptible human beings may be immunized against scarlet fever by the use of hæmolytic streptococcus toxin. In horses a therapeutic antitoxin is produced following the administration of toxin. By the so-called Dick test they have been able to prove the specificity of their virus, and this test has also served in the differential diagnosis of scarlet fever from other exanthematous diseases.

There is evidence that more than one strain of scarlet-fever streptococcus exists, and the typing of these strains will require careful attention in future investigations upon the subject. While the evidence in favor of the hæmolytic streptococcus etiology of scarlet fever is fairly complete, there still remains doubt in some quarters that scarlet fever is caused by a streptococcus and there are still some who regard the streptococcus as a secondary invader and favor the theory that scarlet fever is in reality caused by a filterable virus. The evidence does not support the latter conception. Some strains of streptococci are filterable, and upon this basis it is permissible to consider scarlet fever as one of the filterable group of diseases of bacterial origin but certainly not one of the ultramicroscopic viruses.

Smirnowa-Zamkowa(14) has recently described certain minute oxyphil bodies in the tissues and the bile of persons dead of scarlet fever. These bodies stain with eosin, and cultures that were positive through several transfers when injected into rabbits gave rise to similar bodies in several animal passages. These bodies are apparently attached to the red blood cell and are highly refractile. This author believes that these bodies represent the actual virus of scarlet fever and that they prepare the soil for invasion of the streptococcus universally present in the disease. It will be remembered that Mallory(15) in 1904–05 described certain protozoanlike bodies in four cases of scarlet fever. So-called inclusion bodies in scarlet fever were also described by Hoefer(26) and by Macewen(27) a few years later. Mallory later discarded his protozoan theory of scarlet fever and reported a bacillus (*Bacillus scarlatinæ*) as the cause of the disease. Confirmation of this work has been wanting however.

Stevens and Dochez(16) have recently shown that throat infections with *Streptococcus scarlatinæ* may occur in persons who have previously had scarlet fever, and Nicholls(17) has been able to demonstrate the presence of the infecting agent of this disease three weeks after the onset of the disease. Eagls(18) in England finds that all strains of hæmolytic streptococci from scarlet fever produce toxic filtrates and that these filtrates are neutralized by scarlet-fever horse serum. The relation between the streptococci of scarlet fever and those of erysipelas and puerperal fever is pointed out by this author. Vas(19) has confirmed the specificity of the Dick test for susceptibility to scarlet fever, and Smith and Taylor(20) believe that the Dick test will in time be as firmly established in relation to scarlet fever as the Schick test is in relation to diphtheria. Smith(21) has been able to divide into two types the hæmolytic streptococci obtained from two hundred ten cases of scarlet fever by serological methods. In this study Smith found that type I was present in one hundred nineteen cases and type II was obtained from fifty-seven cases. This author isolated streptococci from 92 per cent of two hundred ten cases.

Regardless of the current doubts upon the streptococcus etiology of scarlet fever we must conclude that hæmolytic streptococci appear to be the true cause of this disease. Pertinent experimental data will be necessary to discredit this.

Incubation period of scarlet fever.—In 87 per cent of one hundred thirteen carefully selected cases Holt(22) has determined the incubation period of scarlet fever as ranging between two and six days. He states that "speaking generally if, after exposure, a week passes without symptoms, the chances of infection are very small."

Symptoms of scarlet fever.—As a rule the onset of the disease is abrupt. In most cases there is vomiting, rapid rise in temperature, and sore throat. In severe cases the temperature rises to 104 or 105° F., but in mild cases it may not exceed 101° F. Usually the throat presents evidences of simple inflammation, but in some cases there may be membranous patches. The eruption appears from twelve to thirty-six hours after the first symptoms begin. In some cases the eruption does not appear for four or five days, but this is rare. The rash is fully developed within twenty-four hours after its appearance. It persists from three to seven days. The rash appears first upon the neck and chest, and consists of very minute points upon a reddish background giving the appearance of a uniform blush. Later the rash covers the entire body except the face, and even in severe cases the central part of the face escapes. Often the rash is too faint to be easily recognized. A well-developed bright rash usually indicates strong heart action, and a sudden recession of the rash is a sign of heart failure. (Holt.)

The rash is accompanied by intense itching and burning, and in some cases marked swelling of the hands and the face. On or about the eighth day shortly after the rash has faded, there is noted an exfoliation of the dead epidermis. This is spoken of as the stage of desquamation. Desquamation may last from three to seven weeks or longer. The chief complications of scarlet fever are otitis, adenitis, membranous inflammations of the pharynx or larynx, and nephritis. Under five years of age the average mortality from scarlet fever is between 20 and 30 per cent.

Animals susceptible to scarlet-fever virus.—Man is the natural host for the infectious agent of this disease. Experimental scarlet fever in other mammals has been very unsatisfactory. None has been found that when inoculated with material from cases of the disease will develop symptoms typical of scarlet fever.

Immunity.—As a rule one attack of scarlet fever confers immunity for life. Second attacks have been observed in a few isolated instances in which the observation is thought to be beyond question. Relapses and recurrences within a short time after an attack of the disease also occur, but before sufficient time has elapsed to have established a high degree of immunity. That there is a natural and racial immunity to scarlet fever is indicated by its relative absence from certain parts of the world, notably the Tropics. According to Sherwood, Noble, Nigg, and Baumgartner(23) American Indians have a high degree of natural immunity to scarlet fever, although they are susceptible to other exanthematous diseases. While skin tests indicated that during the early years Indians are apparently as susceptible to scarlet fever as white children, as the Indian reaches maturity his immunity is much greater. Human beings may be immunized against scarlet fever by the streptococcus toxin elaborated by hæmolytic streptococci isolated from cases of the disease. Antitoxin has been produced in horses and possesses a definite therapeutic value.

Pathology.—The pathology in uncomplicated cases of scarlet fever is limited to the throat and skin. Except in the hæmorrhagic form, the skin after death shows no traces of the rash. There are no specific lessions. (Osler.) In the throat the changes consist of simple inflammation, follicular tonsillitis, and in extreme cases, diphtheroid angina. There may be lymphadenitis and œdema of the tissues of the neck. The lymph glands may show hyperplasia, and the spleen, liver, and kidneys evidences of focal necrosis. Pericarditis and endocarditis occur as well as myocarditis in some cases. Other anatomical changes depend largely upon complications and are not a part of uncomplicated scarlet fever.

Prevention.—Prevention of scarlet fever depends upon early diagnosis, isolation, and prophylaxis. All cases of this disease should be isolated. Susceptible individuals may be immunized with antitoxin (passive immunity) or with toxin (active immunity). Convalescent human serum may also be employed for immunization (passive immunity). The Dick test should be applied to exposed cases and for diagnosis in doubtful cases.

The Dick test is quite similar to the Schick test. A small quantity of the specific hæmolytic streptococcus toxin is injected into the skin. This amount is usually 0.1 cubic centimeter in quantity of a dilution of toxin which produces a typical reaction. Susceptibility is indicated by a positive reaction, while a negative reaction signifies immunity to scarlet fever. The reaction appears in about six hours and is fully developed eighteen to thirty-six hours later. It consists of a circular area of redness, and swelling in some cases. It usually subsides very rapidly. Pseudoreactions occur that are very difficult to distinguish from true reactions. This test possesses definite diagnostic value.

For the production of active immunity it is recommended by Young and Orr(24) that three injections of toxin be given beginning with 500 skin doses for the first injection, 5,000 for the second, and 30,000 skin test doses for the third. The injections are given at intervals of two weeks. Immunity produced in this way is believed to last about two years. Passive immunity, while of value, is of very short duration and is not believed to persist more than a few weeks.

Isolation of cases of scarlet fever should be established for at least three weeks after the onset of symptoms, and in most cities of the United States the regulations require thirty days. Kanevskaya(25) has recently demonstrated the presence of streptococci in the scales during the stage of desquamation in scarlet fever. In thirty cases this author found hæmolytic streptococci and states that by the thirtieth day of the disease the streptococci have disappeared from the scales in most cases. He is inclined to the view that the streptococci in the scales have their origin not in the skin, but in the blood stream. If this be true it would appear that health units should advocate at least a 30-day period of detention for these cases. Hospitalization, closing of schools in severe epidemics, Pasteurization of milk, good nursing, and proper disinfection will all contribute to the lowering of the mortality and spread of scarlet fever.

BIBLIOGRAPHY

1. DICK and DICK, Journ. Am. Med. Assoc. 77 (1921–1924) 782; **81** (1921–1924) 1166; **82** (1921–1924) 265, 301, 542.
2. VAUGHAN, Epidemiology and Public Health. Mosby & Co., St. Louis (1922).

3. KLEIN, Rep. Med. Off., Local Govt. Ed. London (1896–7) 263.
4. BAGINSKY and SOMMERFELD, Berl. klin. Wchnschr. 37 (1900) 588.
5. MOSER, Wien. klin. Wchnschr. 15 (1902) 1302.
6. SCHICK, Deutsch. med. Wchnschr. (Dec. 1905).
7. SAVCHENKO, Russk. Vrach. 4 (1905) 797.
8. GABRITSCHEWSKY, Russk. Vrach. 10 (1906) 467; Berl. klin. Wchnschr. 44 (1907) 556.
9. SCHULTZ and CHARLTON, Ztschr. f. Kinderh. 17 (1918) 328.
10. BLAKE, Lancet, London 2 (1927) 495.
11. DOCHEZ and BLISS, Journ. Am. Med. Assoc. 74 (1920) 1600.
12. TUNNICLIFF, Journ. Am. Med. Assoc. 74 (1920) 1368.
13. DOCHEZ and SHERMAN, Journ. Am. Med. Assoc. 82 (1924) 542.
14. SMIRNOWA-ZAMKOWA, Virchow's Arch. f. path. Anat. 261 (1926) 821.
15. MALLORY, Journ. Med. Res. 10 (1904–05) 483.
16. STEVENS and DOCHEZ, Journ. Am. Med. Assoc. 87 (1926) 2137.
17. NICHOLLS, Am. Journ. Hyg. 7 (1927) 84.
18. EAGLES, Brit. Journ. Exp. Path. 7 (1926) 286.
19. VAS, Klin. Wchnschr. 5 (1926) 1232.
20. SMITH and TAYLOR, Journ. Hyg. 25 (1926) 90.
21. SMITH, Journ. Hyg. 25 (1926) 165.
22. HOLT, Diseases of Infancy and Childhood. Appleton & Co., New York and London (1920).
23. SHERWOOD, NOBLE, NIGG, and BAUMGARTNER, Journ. Immunology 11 (1926) 343–360.
24. YOUNG and ORR, Journ. Am. Med. Assoc. 86 (1926) 1340.
25. KANEVSKAYA, Mikrobiologicheskiy Jurnal, Leningrad 4 (1927) 209.
26. HOEFER, Deutsch. med. Wchnschr. 37 (1911) 1063.
27. MACEWEN, Journ. Path. and Bact. 18 (1913–14) 456.

OROYA FEVER AND VERRUGA PERUVIANA: CARRION'S DISEASE

Definition.—Oroya fever and verruga Peruviana are apparently the manifestations of one and the same disease. This disease is characterized by an acute specific fever, rapidly developing anæmia of the pernicious type, tenderness over the blood-forming tissues, and a granulomatous eruption of extreme chronicity which is associated with the fever, the joint pains, and hæmorrhages.

History and distribution.—This disease has long been endemic in certain valleys of the Andes in Peru. It is said to exist between the ninth and sixteenth parallels of south latitude, and at an elevation of from 3,000 to 10,000 feet on the western slopes of the Andes. Apparently the disease is confined to certain hot, narrow valleys, the inhabitants of neighboring places being exempt. (Manson.) According to the natives in this region the disease may be acquired by merely passing through the endemic districts, and especially if the traveller passes the night there. In 1885 Carrion, who thought the disease verruga

Peruviana was communicable, inoculated himself and died a month later with a febrile disease that is now known to have been Oroya fever. The disease has since been designated Carrion's disease in honor of this investigator who gave his life to it. During the last century it was thought that these two conditions were only different manifestations of the same disease, the verruga being a later stage of Oroya fever. This opinion held sway until 1913 to 1915 when Strong(1) and his associates on the Harvard Commission published the report of their expedition to South America. In this report the commission reported their experiments with verruga Peruviana in which a volunteer was inoculated with verruga Peruviana material and sixteen days later developed verrucose lesions without the fever or anæmia that is characteristic of Oroya fever. They concluded that the two conditions were not the same disease, since each differs from the other markedly in its clinical manifestations. The commission recognized that the two conditions are endemic in the same localities and suggested that the two conditions are found in the same individual, suggesting in this way a common etiology.

It appears now that these two conditions are merely manifestations of the same disease, and strong evidence to support this view has been presented recently by Noguchi.(2)

The virus of Oroya fever and verruga Peruviana.—In 1909 Barton(3) described small rodlike bodies in the red blood cells and in endothelial cells of the lymphatic glands from cases of Oroya fever. These bodies were regarded by Barton as of protozoal origin and were later termed by the Harvard Commission *Bartonella bacilliformis.* Strong has regarded them as protista, related to *Grahamella,* and the probable cause of the disease. Odriozola(4) had associated these two conditions in 1896 and stated that Oroya fever may appear in a patient having verruga Peruviana, or verruga Peruviana may occur in a patient having Oroya fever, or the two may occur in the same patient simultaneously. The Harvard Commission found that they could transmit verruga Peruviana from man to man and to monkeys by rubbing into the eyebrow the material from a verruga granuloma. In 1913 da Rocha Lima(5) reported the presence of minute granules in the endothelial cells of the granulomatous lesions which could be stained by the Giemsa or the Levaditi method. These granules were regarded as Chlamydozoa-like bodies, and since such inclusion bodies have been demonstrated in several filterable virus diseases, the possibility of the filterable nature of the causative agent in verruga Peruviana was suggested. The work of the Harvard Commission, however, indicates that the causative agent of this condition is too large to pass through a filter, since they found their filtrates noninfective. Strong has also shown that there is a cloudiness produced in tubes of Noguchi's medium inoculated with verruga material and further that he is able artificially to immunize monkeys against this disease.

Bartonella bacilliformis somewhat resembles a piroplasm, *Theileria parva,* as it is seen during its life cycle in the lymphatic gland, and in blood smears these organisms may be very numerous. They occur as rod-shaped bodies and as rounded, oval, or pear-shaped bodies, which stain an intense blue with Romanowsky. Frequently chains and branching forms are observed.

In 1926–27 Noguchi published a series of papers dealing with the etiology of these two conditions. From the blood of a patient having Oroya fever, who later died of the disease, Noguchi obtained a pure culture of *Bartonella bacilliformis,* which he grew on semisolid *Leptospira* medium and also on slant agar containing animal blood. The organism is an obligate aërobe, is Gram negative, and under certain conditions is motile. Intravenous injection of cultures of this organism into young macaques, according to Noguchi, induces a prolonged irregularly intermittent fever. The organism is detected in the blood corpuscles of the injected monkey and Noguchi states "reproducing the precise appearances observed in human cases of Oroya fever." The intradermal inoculation of this culture into the eyebrow of young macaques induces nodular formations, which are rich in new blood vessels and the bacilliform organism is found within the endothelial cells. The organism can be cultured from these nodules. According to this author *Bartonella bacilliformis* resists 4° C. for one hundred fifty-two days; it remains viable in the excised nodule for fifty-six days at 4° C. and for twenty-eight days at room temperature; the organism remains motile for about two weeks in a suitable medium. In other experiments Noguchi demonstrated that the symptoms and the lesions produced in the chimpanzee and orang-utan with *Bartonella bacilliformis* are far milder than those produced in rhesus monkeys and are not as characteristic of Oroya fever and verruga as it occurs in human beings. This author also succeeded in transmitting the infection by means of a tick, *Dermacentor andersoni,* to normal rhesus monkeys and recovered the infecting organism from the lymph nodes and blood of the infected animals. In 1927 Noguchi reported that monkeys, which had recovered from the Oroya strain of *Bartonella bacilliformis,* were found immune when tested with the verruga strain of *Bartonella bacilliformis* as well as against the homologous strain.

These experiments of Noguchi indicate that Oroya fever and veruga Peruviana are one and the same disease and that the character of the disease produced in monkeys depends upon the route of inoculation; further, that the cause of the disease is *Bartonella bacilliformis* and that this organism can be isolated from either Oroya fever when it exists alone or from verruga Peruviana when it is the only indication of the disease. Cross-immunity experiments have established the identity of the microbe obtained from each type of the disease.

While these experiments apparently settle the question of the etiology of Oroya fever and verruga Peruviana, thorough study on the filtrability of this agent has not been made to a point where it can be said definitely that filterable forms of the virus do not exist. It is probable that with confirmation of this notable piece of work this disease will be eliminated from the list of diseases known or thought to be caused by filterable agents. For the present it has been thought wise to include this disease in the present review.

There is some indication that Oroya fever is transmitted to man through the agency of some insect, but except for the experimental transmission of the disease to monkeys by the tick *Dermacentor andersoni* this theory has not been established.

Incubation period of Oroya fever and verruga Peruviana.—In the natural infection, the form of this disease known as Oroya fever has an incubation period of about three weeks while verruga requires an incubation period ranging from ten to forty-five days. In one of Noguchi's monkeys (monkey 25), in which he obtained the simultaneous occurrence of severe symptoms of both veruga and Oroya fever, the incubation period was fifteen days.

Symptoms of Oroya fever and verruga Peruviana.—That this disease exists in two forms is well recognized. The essential symptoms of Oroya fever are malaise; pains in the head, the joints, and the long bones; an irregular fever; and a rapidly developing anæmia of the pernicious type. In this type of the disease the spleen and the liver are enlarged and tender. The mortality ranges from 10 to 40 per cent, resulting usually within two or three weeks after the onset of symptoms and in some cases ending in delirium. The verruga type of the disease is also characterized by rheumatic pains and initial fever. The eruption is either miliary or nodular, sparse or abundant, discrete or confluent. Usually the eruption begins as a macule, later becoming dark in color and nodular. These nodules may be flat or slightly pedunculated. Individual lesions may become very large and when strangulated become a source of danger from hæmorrhage. The lesions appear both superficially in the skin and also upon mucous membranes (mouth, œsophagus, stomach, intestines, bladder, uterus, and vagina), which may be followed by hæmatemesis, melæna, hæmaturia, and bleeding from the vagina. Dysphagia is a common symptom. This type of the disease may extend over a period of two or three months.

Animals susceptible.—Man is the natural host for the virus of Oroya fever and verruga Peruviana. Monkeys have been experimentally infected, and rabbits and dogs are said to be susceptible.

Immunity.—There is both a natural and an acquired immunity to this disease. Noguchi produced immunity in monkeys to the viruses obtained from cases both of Oroya fever and verruga. Strong was able to produce artificial immunity to verruga in monkeys with graded doses of verrugous material.

Pathology.—The characteristic feature in Oroya fever is the anæmia. The blood count may drop remarkably within three or four days, the blood picture simulating that of pernicious anæmia. At first there is a marked polymorphonuclear leucocytosis with a disapperance of eosinophils, and during the later eruptive stage an eosinophilia with a mononuclear leucocytosis. (Manson.) There are areas of degeneration and central necrosis in the liver and spleen. The lymph glands exhibit large macrophage endothelial cells containing rod-shaped bodies (*Bartonella bacilliformis*). In verruga the changes consist of proliferation of the endothelium of the lymphatic channels, which are filled with plasma cells and fibroblasts. The granulomatous tumors are vascular and prone to bleed profusely. Manson states that "a feature of the pathological histology is the formation round the blood-vessels of nodules or angioblasts characteristic of the disease."

Control measures.—For the prevention of this disease it has been recommended that workmen should quit the endemic locality before sunset. It is said that the case incidence of the disease (Oroya fever) has been lowered in certain localities following this measure. Possibly as a result of recent investigations on the etiology of this disease some method of immunization may be devised. The search for a transmitting insect should be continued, and if found its eradication, of course, would be indicated.

BIBLIOGRAPHY

1. STRONG, TYZZER, SELLARDS, BRUES, and CASTIABURU, Report of first expedition to South America, Harvard School of Tropical Medicine, Cambridge (1913) 1915.
2. NOGUCHI and BATTISTINI, Journ. Exp. Med. 43 (1926) 851–864.
   NOGUCHI, Journ. Exp. Med. 44 (1926) 533–538, 697–713, 715–728, 729–734; 45 (1927) 455-463, 781-786.
3. BARTON, Crón. méd. 26 (1909) 7.
4. ODRIOZOLA, La maladie de Carrion, Paris (1896).
5. DA ROCHA LIMA, Centralbl. f. allgem Pathol. u. pathol. Anat. Verhandl. d. deutsch. path. Gesellsch. zu Marburg 24 (1913) 409.

ANÆMIA OF RATS

Definition.—Anæmia of rats is characterized by a marked decrease in the number of red blood cells and a rapid drop in the hæmoglobin content of the blood. The decrease in the number of red blood cells and the amount of hæmoglobin in the blood may take place within a few days, the affected rats often dying of the malady. Other rats recover, improvement often beginning suddenly with a marked shower of normoblasts. This affection has been thought by some investigators to be caused by a filterable virus and by other authorities is said to be due to an infection with *Bartonella muris.*

The virus of rat anæmia.—It has been shown by a number of investigators that an anæmia develops in white rats following splenectomy. According to Mayer, Borchardt, and Kikuth(1) this anæmia is thought to be due to a latent infection with *Bartonella muris,* which is apparently activated when the animal is splenectomized. These authors found *Bartonella muris* infection in rats to be fairly general in Hamburg. The infection has also been described in Viennese rats by Lauda(2) and by Nauck(3) in Peking. Haan, Lauda, and Sorge(4) have reported that the rats in southern Italy are not infected with organisms of the *Bartonella* group, but when these rats are injected with the organs of Viennese rats before splenectomy, they develop a characteristic anæmia after they have been splenectomized.

Jaffé and Willis(5) in Illinois have recently examined two strains of rats for the presence of *Bartonella muris.* One of these was a standard pedigreed strain from a local dealer. These authors state:

> Of the pedigreed rats all which have so far been tested have proved infected. The Bartonella appeared in the peripheral blood from four to five days after the splenectomy. Within two days the red count then dropped from 6½ millions to 2½ millions and the hemoglobin content decreased from 100 % to 35%. There was a marked neutrophile leukocytosis. Some of the rats died during the severe attack of anemia; some recovered spontaneously. Recovery starts with a sudden and very marked shower of normoblasts.

These authors further demonstrated the interesting fact that young rats of the pedigreed group of about four weeks of age showed numerous bartonellæ in the blood stream before operation. It has been pointed out by Lauda that they are rarely found in the blood stream of adult rats before operation and if present are very few.

Jaffé and Willis found that the rats obtained on the market were apparently not generally infected. Only one rat of this group developed anæmia following splenectomy. Seven rats in all were tested. The rat that developed anæmia recovered completely within three weeks. Two of the rats were starved for three days and showed bartonellæ. One rat died of pneumonia but no bartonellæ were found in the heart's blood. These authors recommend the panoptic blood-staining method of Pappenheim for the detection of bartonellæ. They also employed a slightly alkaline Giemsa solution followed by decolorization in a 1 per cent sodium carbonate (Na_2CO_3) solution. They state that bartonella is first visible between the red blood cells and is composed of a small biscuit-shaped body having two granules held together by a thin capsule. Later these bodies become attached to the surface of the red blood cells and appear as slender rods that stain blue and contain two small purple granules on both ends. Jaffé and Willis are not convinced that the parasites invade the interior of the red blood cells. They state that as many as thirty microörganisms may be attached to a single red blood cell and that a great variety of shapes and sizes may be noted.

It will be recalled that *Bartonella bacilliformis* has been shown by Noguchi and others to be the cause of Oroya fever and verruga Peruviana and the organism has been cultivated by Noguchi upon semisolid leptospira medium and also on slant agar containing animal blood. Anæmia is also the characteristic feature of Oroya fever. *Bartonella muris* has not as yet been cultivated artificially. While *Bartonella bacilliformis* of Oroya fever invades the interior of the erythrocyte, *Bartonella muris* of rat anæmia apparently does not.

While the evidence is not complete, it strongly suggests that at least one form of anæmia of rats is caused by a *Bartonella* infection. Further investigations will be necessary to demonstrate whether or not filterable forms of *Bartonella muris* exist. Furthermore, an investigation of the relation of *Bartonella muris* and *B. bacilliformis* would be exceedingly interesting, since both are associated with diseases characterized by anæmia.

BIBLIOGRAPHY

1. MAYER, BORCHARDT, and KIKUTH, Arch. f. Schiffs- und Trop.-Hyg. Beiheft 31 N. 4 (1927).
2. LAUDA, Wien. med. Wchnsch. 77 (1927) 772.
3. NAUCK, Arch. f. Schiffs- und Trop.-Hyg. 31 (1927) 322.
4. HAAN, LAUDA, and SORGE, Klin. Woch. 6 (1927) 2240.
5. JAFFÉ and WILLIS, Proc. Soc. Exp. Biol. and Med. 25 (1928) 242.

CONTAGIOUS PLEUROPNEUMONIA

LUNG PLAGUE; LUNGENSEUCHE DER RINDER (GERMAN); PERIPNEUMONIE CONTAGIEUSE (FRENCH); POLMONERA, PLEUROPOLMONITE ESSUDATIVA (ITALIAN)

Definition.—Contagious pleuropneumonia is defined by Hutyra and Marek as an acute or subacute, but sometimes chronic contagious disease of cattle, which is characterized by an exudative inflammation of the interlobular lymph vessels, and of the alveolar tissue of the lungs, with a simultaneous, serofibrinous pleurisy.

History.—In 1898 Nocard and Roux(1) discovered and artificially cultivated the causative agent of this disease, which had been known in certain parts of Europe since the middle of the seventeenth century. Former workers have considered the disease a form of typhus in cattle, and some thought of this condition as a paralysis of the lungs. Notable contributions to the etiology of this disease have been made by Bordet,(2) Borrel,(3) and others.

Distribution of the disease.—Contagious pleuropneumonia was known in the seventeenth century in Switzerland and appeared about that time in parts of Germany and France. Later it appeared in Belgium and Holland and spread to Austria and to England in 1841. In 1854 it was carried to South Africa, and in 1843 it spread from England to Sweden and North America. In 1858 it first appeared in Australia. In 1899 the disease appeared in Russia, and in 1910 it became a very serious problem in Spain. About this time the disease spread to Asia, Queensland, New South Wales, Victoria, and South America. The disease has not appeared in the United States since 1892, though it was a serious menace in 1886 in certain states. At present it is prevalent in Russia, Spain, Africa, Australia, and Asia.

Incubation period.—The incubation period following artificial infection (subcutaneous injection of the virus) ranges from six to seven days, following inhalation twelve to sixteen days, and corresponds to the incubation period in the natural infection.

Symptoms of pleuropneumonia.—Under natural conditions the first symptom of the disease is a rise in body temperature of 0.5 to 1°. Almost at once there is loss of appetite and diminished milk secretion. A dry painful cough develops which becomes more painful and more frequent, and in some animals a mucopurulent discharge comes from the nose. As the lung affection progresses, respiration becomes more difficult, the skin loses its elasticity, the hair become rough, constipation develops, and the animal becomes emaciated. Near the end of the disease the fever rises to 41 or 42° C. and a subcutaneous œdema develops over the chest and the abdominal walls. Pregnant animals usually abort. The disease may terminate in death within a week but usually runs for four or five weeks. About half of the cases die of the disease.

Animals susceptible to the virus of pleuropneumonia.—Cattle, buffaloes, reindeer, camels, yak, and bison are all susceptible to the disease. Other animals and man are not susceptible. A form of pleuropneumonia has been described as occurring in goats in the mountain districts of Germany and also in the Pyrenees. It is believed to be the same disease as "boufrida" which occurs in Algeria. No causative organism has been found, and it is not related to pleuropneumonia in cattle although the anatomical changes in the lungs closely resemble this disease.

The virus of pleuropneumonia.—The virus of pleuropneumonia is a small polymorphic organism that is filterable through Berkefeld and Chamberland filters. Under high magnification these organisms appear as small fine vibrios, short spirillæ, and asteroid bodies. According to Bordet on artificial medium the organisms appear as fragile spirochætes but are shorter than the spirochætes of syphilis. Borrel found in stained preparations fork-shaped branchings and asteroid bodies and for this reason designated the organism *"Asterococcus mycoides."* Lipschütz has described the bodies of pleuropneumonia as roundish clumps about 0.25 millimeter in size that occur singly or in pairs and occasionally in short chains. These bodies are Gram negative but stain with Loeffler's flagella stain and by Giemsa's method.

Nocard and Roux have cultivated the virus in broth to which lymph from the lungs is added. The medium and the organism were placed into collodion sacks and sewed within the abdominal cavity of rabbits. The culture required fifteen to twenty days to develop. Such cultures injected into cattle produced the disease. The virus can also be cultivated in Martin's bouillon to which 6 per cent beef or rabbit serum has been added. Cultivation has also been accomplished upon solid medium.

The virus is destroyed within twenty minutes when exposed to a temperature of 60° C. It will survive freezing temperature for several months. The virus is resistant to glycerin and will resist 0.5 per cent phenol for some time. Exposure to light and air quickly destroy it, and cultures lose their virulence within a short time unless hermetically sealed and kept at a low temperature. In the natural infection it is thought that the virus gains entrance into the body by way of the respiratory tract through inhalation.

Immunity in pleuropneumonia.—Affected animals may transmit the disease to healthy animals in all stages of the disease. The virus may remain latent and virulent in the lungs for years after the animal has recovered from the disease. The susceptibility of cattle varies with the breeds. Some breeds are highly resistant, while others are markedly susceptible. One attack of the disease confers an immunity, and such cattle cannot be infected by artificial means.

Vaccination both with pleural exudate from cases of the disease and with cultures of the organism has been practiced, the latter giving the most satisfactory results in France. A fairly efficacious antiserum can be prepared by injecting culture material in vaccinated or recovered animals.

Pathology.—The post-mortem findings are, of course, most pronounced in the lungs. Stages of red hepatization, gray hepatization, areas of necrosis, dilated lymph spaces, and thrombosis of the blood vessels are all found. The pleura may be covered with a fine exudate and be somewhat thickened. The pleural cavity may contain a large amount of clear yellow or turbid exudate, even 15 to 20 liters. Some cases may reveal a fibrinous pericarditis and similar changes of the peritoneum and diaphragm. Finally, there is found a gelatinous infiltration of the subcutaneous tissue over the chest and abdomen.

Control measures.—Isolation and quarantine are strongly advocated, the latter measure for six months or longer. Since the virus remains alive and virulent for long periods after the animals have recovered from the disease, it is recommended that such animals be killed. Laws compelling this procedure have been enacted in several countries. Immunization with pure cultures is also resorted to as a contributing method of control of this disease.

BIBLIOGRAPHY

1. NOCARD and ROUX, A. P. 12 (1898) 240; (1901) 416.
NOCARD, Bull. 158, 203, 317 (1892).
NOCARD, ROUX, and DUJARDIN-BEAUMETZ, Bull. 430 (1899).
2. BORDET, A. P. 24 (1910) 161.
3. BORREL, DUJARDIN-BEAUMETZ, JEANTET, and JOUAN, A. P. 168 (1910).

OTHER REFERENCES

WILLEMS, Rec. (1852) 401; (1887) 11.
MAGENDIE and BOULEY, Rec. (1854) 161.
SUSSDORF, D. Z. f. Tm. 5 (1879) 353.
THIERNESSE and DEGIVE, Ann. (1882) 620.
SCHÜTZ and STEFFEN, A. f. Tk. 15 (1889) 217; 16 (1890) 29; 17 (1891) 290.
MACFADYEAN, Journ. Comp. Path. 5 (1892).
THOMASSEN, Roeckl, Ber. uber d. Kongr. in Bern. (1894) 51.
SCHMIDT, B. t. W. (1898) 159; (1899) 265; Techn. Dept. f. d. Vet. Wesen, A. f. Tk. 25 (1899) 312.
THEILER, Schw. A. 41 (1899) 57.
CONSTANT and MESNARD, Rec. (1903) 436; (1904) 552.
DUJARDIN-BEAUMETZ, A. P. 20 (1906) 449.
MARTZINOWSKI, A. P. 25 (1911) 914.
GROSSO, Hb. d. Serumther (1911) 292.
KELSER, Manual of Veterinary Bacteriology. Williams and Wilkins, Baltimore (1927).
HUTYRA and MAREK, Pathology and Therapy of the Diseases of Domestic Animals, 3d Eng. ed. Mohler and Eichhorn. Alexander Eger, Chicago (1926).

AGALACTIA CONTAGIOSA

Definition.—Agalactia contagiosa is a specific infectious disease of the mammary glands of sheep and goats, that in the lactating female is characterized by an alteration in the milk, which becomes yellowish or brownish in color and salty in taste, and may become purulent; the secretion of milk diminishes and may stop altogether. The lesions may remain localized in the mammary glands, or the infection may provoke other specific lesions. It is thought to be caused by a filterable virus.

History and distribution.—Agalactia is a relatively common disease in many species of animals. In cattle mastitis is caused by a streptococcus.(1) The disease referred to in this section is in no way related to ordinary mastitis in cattle, but is a specific infectious disease of sheep and goats. The disease has been particularly prevalent in Algeria, and studies upon the etiology of the infection have been made with material from cases occurring in that part of the world. In general the disease may be regarded as relatively uncommon. There is no record of its ever having been present in the United States.

The virus.—Celli and De Blasi(2) very early demonstrated that the infective agent in agalactia contagiosum is filterable through Berkefeld and Silbersmith filters. This was confirmed by Carré(3) in 1912. In 1923 Bridré and Donatien(4) reported the transmission of the disease to goats, confirmed the filterability of the virus, and were able to cultivate it in vitro. These authors described a virus in their cultures that resembled forms of both vibrios and spirochætes. These organisms were stained after the method of Giemsa. Under the ultramicroscope numerous granules were found that resembled very small cocci. In some aspects the virus of agalactia contagiosa is similar to the virus of pleuropneumonia. One-half cubic centimeter of culture inoculated into the knee joint of susceptible sheep provokes symptoms of arthritis within four days. The virus is also active in very high dilution. Two goats inoculated under the skin, one with 1 cubic centimeter of culture, the other with 1 cubic centimeter of a dilution of 3:100, developed a specific mastitis eight hours after inoculation.

In 1925 Bridré and Donatien(5) carried their experiments further and confirmed their earlier work. Cultivation of the virus was effected in bouillon to which serum was added in the proportions of 1 : 10 and 1 : 5. Serums from sheep, goats, horses, cattle, man, and rabbits were all found to be satisfactory. It was found that either Martin's peptone or commercial peptone could be used. The addition of some sugars to the medium gave unsatisfactory results, but lactose and mannite favored the growth of the virus. Serum agar was also found to be favorable for the growth of the virus. Stained by Giemsa the virus appears as a very fine microbe taking the violet and appearing in various forms. The usual forms measure from 2 to 5 microns in length, although some are 15 microns in length. The shorter forms resemble vibrios, and some are found to be almost round. Granules were also demonstrated by Bridré and Donatien as in their previous work. The authors state that (translation) "one finds, in a word, with this microbe, all the morphological characteristics described by Bordet, by Borrel, Dujardin-Beaumetz, Jeantet, and Jouan with the microbe of pleuropneumonia." The organisms possess a motility comparable to the movement of mosquito larvæ in water, although these authors believe that this motion is a false movement and that the virus must be considered nonmotile.

Bridré and Donatien state that the virus passes a Chamberland No. 1 filter, but that the filtrate remains sterile when the L 2 Chamberland is employed. This property of the virus permits its isolation with great facility.

The optimum temperature for the virus of agalactia is 37° C., but growth is possible between 24 and 41.5° C. The virus remains virulent after four months when preserved at —5 to —12° C. It is apparently destroyed after five months at such low temperatures. It is destroyed within ten minutes when exposed to 53° C. It resists a temperature of 50° C. for one and one-half hours. Agglutination, precipitation, and deviation of complement experiments have not been entirely satisfactory. Cultures of the organism have been found to be as satisfactory for inducing the infection in sheep and goats as the natural virulent material taken from animals having the disease. Sheep are somewhat less sensitive than goats. The female in lactation is the best animal in which to induce the infection. Inoculation by scarification is followed by a local reaction, which is later followed by the specific infection as in subcutaneous inoculations. Intravenous inoculation of the virus provokes a severe reaction. Inoculation of the virus in cattle induces a local reaction only.

Bridré and Donatien again have pointed out the resemblance of the virus of agalactia to that of pleuropneumonia. Both viruses may be cultivated in serum-bouillon mixtures; the colonies upon serum agar are identical; both viruses are filterable through the same filters; their morphology is practically identical; their resistance to different temperatures is the same. On the other hand the virus of agalactia grows more luxuriantly than the virus of pleuropneumonia and, furthermore, Bridré and Donatien have succeeded in cultivating the virus of agalactia in milk, while the virus of pleuropneumonia does not grow in

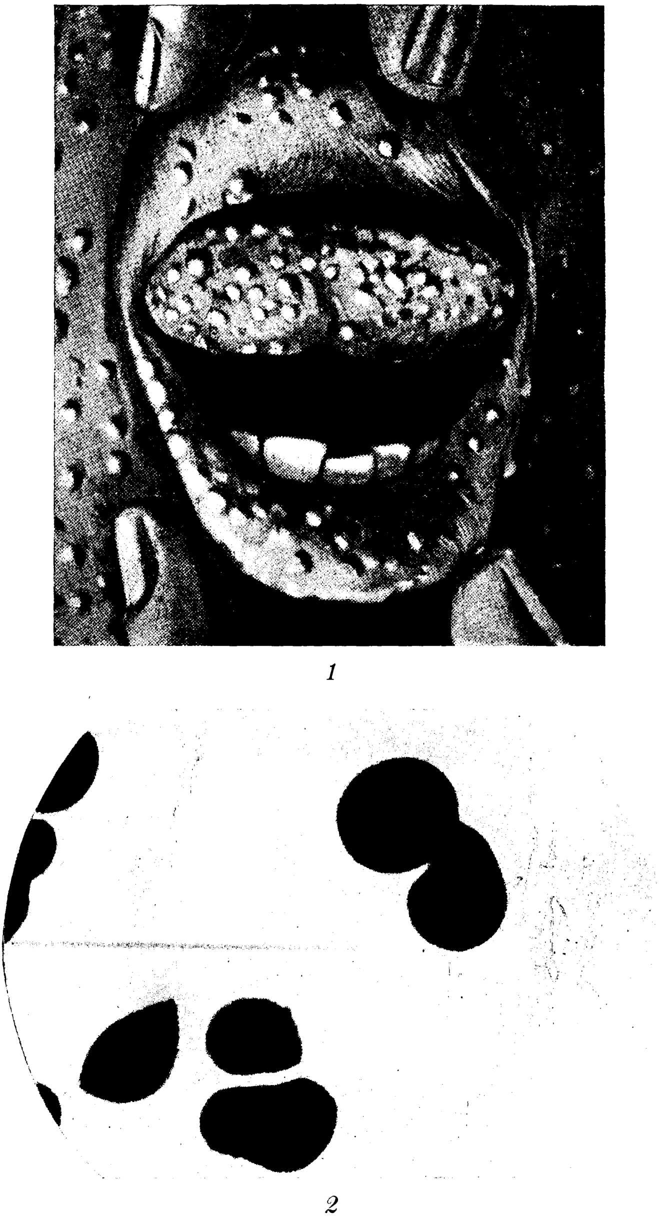

Fig. 1. Verruga Peruviana, showing nodules on skin and mucous membrane of mouth. 2. Bartonella muris, showing parasites overlapping edges of the erythrocytes.

PLATE 13.

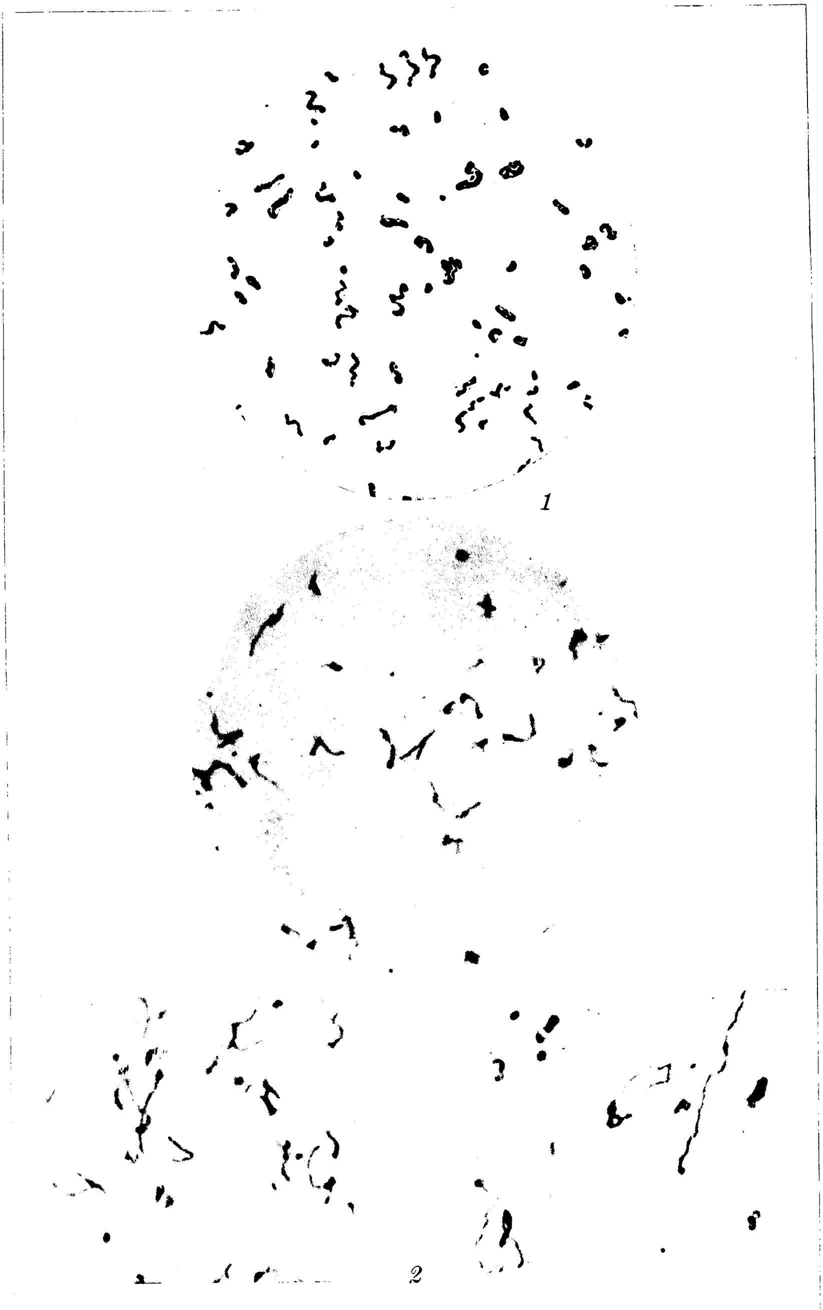

Fig. 1. Asterococcus mycoides of contagious pleuropneumonia. 2. Types of filterable microörganisms found in agalactia contagiosa.

PLATE 14.

this medium. The virus of agalactia is easily isolated from the milk of infected animals.

A preventive serum is obtained from sheep, goats, and horses; 20 cubic centimeters of serum will protect against an infectious dose of culture of the virus. Active immunization of animals in lactation was found to be exceedingly difficult by any method attempted.

The virus of agalactia contagiosum is of special interest to students interested in the filterable virus field. Cultivation of the filterable viruses has proved to be exceedingly difficult, and there are only three or four with which this has been accomplished.

BIBLIOGRAPHY

1. KOLLE and WASSERMANN, Handbuch der pathogenen mikroorganismen. Gustav Fischer, Jena **6** (1913).
   BUCHANAN, Veterinary Bacteriology. W. B. Saunders Co., Philadelphia (1922).
2. CELLI and DE-BLASI, Annali d'Igiene sperimentale, fasc. 2 (1906). Prima esperienze di vaccinazione contre l'agalassia contagiosa delle pecore e capre, brochure, Milan.
3. CARRÉ, Annales **26** (1912) 937.
4. BRIDRÉ and DONATIEN, C. R. de l'Acad. des Sci. **177** (1923) 841; Bull. de la Soc. de méd. Vét. (Nov., 1923) 441.
5. BRIDRÉ and DONATIEN, Ann. l'Inst. Pasteur **39** (1925) 925.

ILLUSTRATIONS

PLATE 13

FIG. 1. Verruga Peruviana, showing nodules on skin and mucous membrane of mouth. (After Biffi; from Manson.)

2. *Bartonella muris*, showing parasites overlapping edges of the erythrocytes. (After Jaffé and Willis.)

PLATE 14

FIG. 1. *Asterococcus mycoides* of contagious pleuropneumonia. (After Hutyra and Marek.)

2. Types of filterable microörganisms found in agalactia contagiosa, from bouillon-serum cultures. (After Bridré and Donatien.)

CHAPTER VI

INSECT-BORNE DISEASES OF MAN AND ANIMALS (FILTERABLE)

YELLOW FEVER

TYPHUS ICTEROIDES; FIEBRE AMARILLA

Definition.—Yellow fever is an acute, specific, febrile disease which occurs in epidemic or endemic form, usually within a limited geographic area. It is characterized by fever, chills, marked muscular pain, evidences of blood destruction, black vomit, albuminuria, and hæmatogenous jaundice. The disease is caused by a filterable virus and is transmitted to man by the mosquito *Aëdes ægypti.*

History and distribution.—The first authentic description of yellow fever was given by du Tertre, who described the disease in Guadaloupe in 1635. According to Vaughan(1) this disease was confined to the Western Hemisphere before connection between the two hemispheres was established by Columbus. Since the beginning of the seventeenth century epidemics of yellow fever have been described. Such epidemics have been particularly severe in the West Indies and in parts of Central America. Following the abolition of the quarantine of European ports during the French Revolution, the disease during the period of 1791 to 1815 spread into Europe and to both North and South America. Later we find records of the disease in Cuba, Jamaica, Santo Domingo, Brazil, and Porto Rico. The disease was endemic in these parts of the world until the close of the nineteenth century. From the West Indies the disease spread northward through Mexico to the southern United States and southward as far as Montevideo. The disease was common on the west coast of South America in Peru, Chile, and Colombia and spread to the Gulf of California from the Isthmus of Panama. During 1923 the disease was stamped out of Peru and at present is only endemic in a few areas in Central and South America and in Brazil.

Yellow fever is endemic on the West Coast of Africa. In 1922 there was an epidemic on the French Ivory Coast, and one in

Southern Nigeria and the Gold Coast in 1923. At present a commission is studying the disease on the Gold Coast in an effort to bring about its final eradication.

The virus of yellow fever.—In 1900 General Sternberg appointed a commission to study the transmission of yellow fever in Cuba. The transmission of yellow fever through the agency of the mosquito had been forecasted by Finlay in 1891. The notable achievement of Reed, Carroll, and Agramonte(2) in 1901 in establishing the scientific facts that proved this theory is a landmark in medical science. Lazear, who was also a member of this commission, died of the disease in the course of the experiments. This commission proved beyond all doubt that yellow fever is transmitted to man through the bite of the mosquito *Aëdes ægypti.* While the mode of the transmission of the disease was discovered by this commission, the actual causative agent of yellow fever remained obscure.

In 1919 Noguchi(3) reported that he had isolated the specific cause of yellow fever in Guayaquil, in the form of a spirochæte which he named *Leptospira icteroides.* He found that guinea pigs injected with 5 cubic centimeters or more of blood from yellow-fever cases developed symptoms comparable to those of yellow fever in man. Under dark-field illumination Noguchi found a number of spirochætes, similar to *Leptospira icterohæmorrhagiæ,* in the blood, liver, and kidneys. Later he obtained a pure culture of these spirochætes in artificial media and was able to transmit the disease serially through guinea pigs. This organism was found to grow best under aërobic conditions in solid media containing blood serum and at a temperature ranging from 25 to 33° C. He has described this spirochæte as an extremely delicate filament about 4 to 9 microns in length and 0.2 micron in width. It tapers gradually toward the extremities and ends in sharpened points. It is actively motile, shows vibratory motion, and is remarkably flexible. It stains with Giemsa and other polychromatic dyes. Noguchi used for his original source of material one hundred seventy-two cases which were thought by him to be cases of yellow fever. This is a very important consideration in view of the recent work of Sellards. (4) Yellow fever is similar in its clinical manifestations to infectious jaundice, or Weil's disease, the cause of which is a spirochæte similar to the organism described by Noguchi for yellow fever. Of twenty-seven cases Noguchi found the leptospira present in the blood of only three. Monkeys, with the exception of marmosets (*Midas œdipus* or *geoffroyi*), injected with infected blood and tissues were found to be negative. Guinea pigs infected with the blood of presumably yellow-fever cases developed symptoms in from three to six days. These symptoms consisted of a rise in temperature, conjunctival congestion, leucocytosis followed by leucopenia, jaundice, and in some instances hæmorrhages from the nose and anus. The tissues were found at autopsy to be jaundiced and the organs hyperæmic. Hæmorrhages were also noted in the lungs and mucous membranes of the intestines. Noguchi also found that about 67 per cent of the wild rats in Guayaquil showed organisms in their kidneys closely resembling the leptospira found in yellow-fever cases, and when these organisms were injected into guinea pigs similar symptoms and lesions were produced as in the case of the so-called yellow-fever spirochæte.

It is Noguchi's contention that the yellow-fever spirochæte differs from the spirochæte of infectious jaundice. This statement is based upon serological studies. Furthermore, he states that the two spirochætes differ in their pathological properties. The former he states is essentially icteronephritic and produces fatty degeneration of the liver and kidneys, while the spirochæte of infectious jaundice produces jaundice as its chief manifestation. The virulence of Noguchi's so-called yellow-fever spirochæte varies a great deal, but some strains were found infective in doses as small as 0.0001 cubic centimeter of culture. This organism is killed at 55° C. within ten minutes; also by desiccation and freezing. It is destroyed by bile salts, 2 per cent phenol, and 0.1 per cent corrosive sublimate. Later work on yellow fever by Noguchi and Kligler(5) in Yucatan confirmed Noguchi's previous work in Guayaquil.

In 1927 Sellards examined the serum of eleven patients about three and a half months after recovery from typical yellow fever. Sellards found that these serums gave negative Pfeiffer's reactions to both *Leptospira icteroides* and *L. icterohæmorrhagiæ*. This investigator found that two guinea pigs immunized to *Leptospira icterohæmorrhagiæ* gave positive Pfeiffer reactions to both *Leptospira icteroides* and *L. icterohæmorrhagiæ*. This evidence is advanced as additional proof of the identity of these two organisms.

While in the minds of many, Noguchi's work has never been accepted as the final solution of yellow fever, the work of Sellards suggests just cause for extreme conservatism in the question. Reed, Carroll, Lazear, and Agramonte in their early work pointed out that their experiments suggested a filterable agent as the cause of yellow fever. These investigators demonstrated that the infectious agent was present in the blood of patients during the first three days of the disease, and was capable of passing through the pores of a Berkefeld filter. The later work of Noguchi threw doubt upon these experiments, but it now appears that this theory should receive more-serious consideration. At present opinion is divided, and there are few willing to take a stand in the matter; this is perhaps as it should be in view of the present chaos surrounding the question of the etiology of this disease.

In general it may be said that clinically Weil's disease and yellow fever are similar. Especially is this true of the milder cases of yellow fever. Furthermore, the spirochæte described by Noguchi as the cause of yellow fever is morphologically similar to the spirochæte of infectious jaundice, or Weil's disease. The symptoms produced in guinea pigs by both spirochætes are practically identical. Studied by serological methods both spirochætes are somewhat similar even in Noguchi's hands. According to Sellard's experiments the serums of yellow-fever patients do not give a positive Pfeiffer's reaction for either spirochæte. Yet, in Sellards's hands, guinea pigs immune to the spirochæte of Weil's disease give serums that produce positive Pfeiffer's reaction for both spirochætes. Certainly, upon such data it is impossible at the present time to accept *L. icteroides* as the cause of yellow fever.

Leptospira icterohemorrhagiæ is found in the kidneys of a large portion of wild rats in certain parts of the world. In Japan(6) nearly 50 per cent of wild rats are infected; and infectious jaundice is prevalent each year, particularly from September to November. In the United States Jobling and Eggstein(7) found 10 per cent of the wild rats caught in Nashville infected with this organism. In Baltimore Walch and Walch-Sorgdrager(8) found about 33 per cent of rats infected; Langworthy and Moore(9) report 40 to 60 per cent for Albany; Brill(10) finds about 20 per cent of field mice positive in flooded districts of Germany. In a recent study of wild rats, caught in and around Manila, in the Philippines, we have been able to find only one rat out of two hundred fifty examined infected with this spirochæte. While no case of Weil's disease is on record in the Philippines, these results appear unusual in view of the great amount of international shipping, especially between the Philippines and Japan and Formosa where infectious jaundice is prevalent. Recently Cushing(11) has reported in New York City two cases of infectious jaundice. In both cases cultures from the urine yielded the organism and in one case a culture from the cerebrospinal fluid yielded the organism.

Considering the wide-spread distribution of the spirochæte of Weil's(12) disease there can be no question that this is a disease sui generis. That it resembles in its clinical manifestations mild cases of yellow fever is unfortunate, particularly in view of studying the etiology of the latter. That its causative agent so closely resembles the spirochæte described by Noguchi as the cause of yellow fever is also unfortunate, and it should be apparent that we must look elsewhere, probably to the field of the filterable viruses, for the real cause of yellow fever.

If reports are correct the Yellow Fever Commission of the International Health Division of the Rockefeller Foundation on the Gold Coast has recently transmitted yellow fever to monkeys. In a preliminary note Stockes, Bauer, and Hudson(13) report that they have been able to infect monkeys (*Macacus rhesus*) with blood from yellow-fever patients. The first monkey inoculated in this manner died in five days. The virus has been carried from monkey to monkey by inoculation of blood or serum thirty times. The disease transmitted resulted fatally in every case except one. *Aëdes ægypti* mosquitoes became infected when allowed to feed upon infected monkeys and were then able to transmit the disease to normal animals. The mosquitoes were infective in about sixteen days and remained so until death. Serum of infected monkeys filtered through Berkefeld V and N filters and through the Seitz asbestos filter contains the virus and is capable of transmitting the disease to healthy monkeys. These authors have further demonstrated that 0.1 cubic centimeter of convalescent serum from a case of yellow fever protects monkeys against fatal doses of virulent blood as well as against the bites of infected mosquitoes. No leptospira has been found in the body fluids or tissues. It is to be hoped that the work now in progress will result in the discovery of the true etiology of this disease and suggest methods of attack for its final eradication.*

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* Since the preparation of this review several other papers have appeared in the literature by the yellow-fever commission working in Africa. These papers are to be found in the American Journal of Tropical Medicine and the Journal of the American Medical Association for 1928.

Incubation period.—The incubation period of yellow fever ranges from four to thirteen days. Usually it is four or five days but is said to be shorter in some instances. Manson(14) states that its extreme limits are one to fifteen days in the temperate zones, and one to thirty days in the Tropics. Epidemics have been known to develop two weeks after the arrival of a ship in port bringing yellow-fever patients with it.

Symptoms.—As in other febrile diseases the symptoms may develop suddenly with a rise in temperature and chills or there may be prodromal symptoms consisting of general malaise leading up to the more acute symptoms. Usually the symptoms of the disease in uncomplicated cases may be divided into three stages: The initial fever, which may or may not be accompanied by chills; the so-called period of calm; and, in the most severe cases, the period of reaction. The initial fever is usually sudden, the maximum temperature occurring within the first twenty-four hours. The temperature may rise to 103 or 104° F. Following the initial rise in temperature there is a steady drop to 98 or 99° F. In some cases the high temperature may persist for several days and may not reach its maximum until the seventh or eighth day. With the rise in temperature, or soon after, there is usually a chill, headache, supraorbital pain, and pain in the eyeballs associated with photophobia. There may be loin pain, pain in the back, legs, knees, calves, and ankles. Epigastric pain may be a prominent symptom. The skin is dry, the face flushed and swollen. The pulse ranges from 100 to 120 during the first stage but falls to 30 or 40 during the period of calm. This has been designated Faget's sign. Later the tongue becomes coated, dry, and pointed, and thirst becomes intolerable. The palate is congested as are the gums, the latter often bleeding profusely. The eyes become congested and sunken, and by the third day the scleræ become yellowish. Often the skin assumes a yellowish tinge, but in some cases this change is entirely absent. When present the color ranges in intensity from a saffron tint to deep mahogany brown. In fatal cases the yellowish color of the skin is always present, particularly after death. There is practically always a supression of urine and the appearance of albuminuria. The more pronounced these symptoms, the graver the prognosis. The urine is acid and contains casts and blood. The patient may suffer from insomnia, especially after the third day of the disease. Delirium may occur. In severe cases coma may come on near the end of the illness just preceding death. Nausea and vomiting are common. Black vomit is usually regarded as a grave symptom. Such vomited material consists of disintegrated red blood corpuscles and hæmoglobin. It is markedly acid. In some cases the stomach contains pure blood. Hæmorrhages may also occur in other parts of the body, from the nose, mouth, bladder, uterus, and anus. As a rule there is no anæmia and there is first a slight leucocytosis followed later by leucopenia. Death may occur early in the course of the disease, but usually occurs on the fifth or sixth day. The mortality may reach 80 per cent, but usually does not exceed 30 per cent. Among natives in endemic areas it may be lower, ranging from 7 to 10 per cent only.

Animals susceptible.—Man is the natural host for the virus of yellow fever. According to reports from the Gold Coast the commission now studying yellow fever has been able to infect monkeys. According to Noguchi's work *Leptospira icteroides* is infective for guinea pigs.

Immunity.—The immunity produced by one attack of yellow fever is usually permanent. Relapses are rare and are dangerous when they occur.

Transmission.—As stated before yellow fever is transmitted to man by the bite of a mosquito, *Aëdes ægypti.* Manson states that yellow fever may be regarded as a "place disease" like malaria. The disease usually occurs in low-lying, hot, insanitary districts near the wharves and docks of seaport towns. However, it has occurred at high elevations (Sao Paulo, Brazil, 2,500 feet). The disease also follows the rivers and the flat delta country. According to some investigators the virus requires twelve days incubation in the body of the mosquito before the disease can be transmitted to man. Reed and Carroll found *Aëdes ægypti* breeding chiefly in rain-water barrels, sagging gutters, tin cans, cesspools, horse troughs, and in water at the base of leaves. As a rule this mosquito does not fly long distances and is found chiefly around and in the houses in endemic areas. The mosquitoes of this species copulate during the day time, but no eggs are produced until the female has had a meal of blood. Usually the female deposits her eggs upon or near the surface of the water within a week after a meal of blood. The larvæ rest usually near the top of the water but are considered to be bottom feeders. The story of the eradication of this mosquito in Panama by General Gorgas is well known and needs no further elucidation here. The transmission of dengue fever by the same species of mosquito has been described elsewhere as well as the relation between these two diseases. (See Dengue Fever.)

Pathology.—The anatomical changes in yellow fever may be summed up as follows: The pigmented skin is more pronounced in the more dependent parts of the cadaver, due to diffused hæmoglobin rather than to bile pigments; petechiæ are common in the skin and membranes; the tissues of the body and brain are hyperæmic and may show minute hæmorrhages; all the tissues, including cartilage, are jaundiced; red blood cells appear normal; the blood in the vessels is not firmly coagulated; there is generalized fatty degeneration of the capillaries and small blood vessels as well as the liver; the stomach and intestines may contain a dark acid material and the mucous membranes are swollen and show patches of ecchymosis; the spleen shows little change; the kidneys show signs of a parenchymatous nephritis, the renal epithelium showing cloudy swelling and fatty degeneration; lime casts in the kidney tubules have been regarded as characteristic of this disease.

Control measures.—The control of yellow fever rests upon the eradication of the vector, *Aëdes ægypti.* Without this mosquito the disease cannot spread. Watertanks, cisterns, cesspools, etc., should be properly screened; water containers, such as tin cans, water barrels, etc., destroyed or so protected that mosquitoes cannot gain access; and in general the usual methods of preventing the breeding of mosquitoes should be employed. Quarantine and isolation are of course indicated in endemic and epidemic areas.

No immune serum, except that prepared by Noguchi with *Leptospira icteroides,* is as yet available for immunizing purposes.

BIBLIOGRAPHY

1. VAUGHAN, Epidemiology and Public Health. C. V. Mosby & Co. (1922) (ref.).
2. UNITED STATES ARMY COMMISSION, Proc. 28th Ann. Meeting Am. Pub. Health Assoc. (1900); Philadelphia Med. Journ. (1900); Journ. Am. Med. Assoc. (Feb. 16, 1901); (July 6, 1902); (Feb. 22, 1902).
3. NOGUCHI, Journ. Exp. Med. **29** (1919) 547, 565, 585; **30** (1919) 1, 9, 13, 87, 95, 401; **31** (1920) 135, 159.
4. SELLARDS, Am. Journ. Trop. Med. **7** (1927) 71.
5. NOGUCHI and KLIGLER, Journ. Exp. Med. **32** (1920) 601.
6. INADA, IDO, HOKI, KANEKO, and ITO, Journ. Exp. Med. **23** (1926) 377.
   INADA and IDO, Journ. Exp. Med. **24** (1916) 465.
   INADA, Journ. Exp. Med. **26** (1917) 355.
7. JOBLING and EGGSTEIN, Journ. Am. Med. Assoc. **69** (1917) 1787.

8. WALCH and WALCH-SORGDRAGER, Am. Journ. Hyg. 7 (1927) 393.
9. LANGWORTHY and MOORE, Journ. Infect. Dis. 41 (1927) 70.
10. BRILL, Münchener med. Wochenschrift 74 (1927) 1537.
11. CUSHING, Journ. Am. Med. Assoc. 89 (1927) 1041.
12. WEIL, Deutsches Arch. f. klin. Med. 39 (1886) 209.
13. STOKES, BAUER, and HUDSON, Journ. Am. Med. Assoc. 90 (1928) 253.
14. MANSON, Manson's Tropical Diseases. Cassell & Co., ltd., London, 8th ed. (1925).

DENGUE FEVER

Definition.—Dengue is an acute infectious disease transmitted from man to man by the mosquito *Aëdes ægypti* (Linnæus). It is characterized by its sudden onset of fever and malaise and in a large percentage of cases a mild scarlatiniform or measleslike rash.

History.—We are indebted to Siler, Hall, and Hitchens(1) for a recent and critical study of the epidemiology and experimental transmission of this disease in the Philippines in 1924 and 1925. These authors have prepared an exhaustive treatise on the subject of dengue including its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention.

According to Nothnagel(2) the exact etymologic origin of the word "dengue" is unknown. It has been thought in turn to have originated from old Arabic, East African (dinga), Indian (dangue), and Spanish (denguero, meaning "affected" or "dandy-like"). In various parts of the world this disease is known by different names; for example, in Brazil it is called "polka fever;" in the English colonies "pantomime fever;" in America "broken wing" or "breakbone fever;" in the Dutch colonies "knockelkoorts" and "ankle fever;" in Arabia, Syria, Egypt, and Tripoli it is known as "abou-abous," "abou-rekabe," "knee pain," "pére des genoux," and "des massues." Because of the rash it has been known as "fiévre rouge," "calentura roja," "rosalia," "colorado," "giraffe," and "bouquet." A common appellation for many years for this disease has been "scarlatina rheumatica" and at least twenty other names have been employed to designate it in different parts of the world at different times.

Nothnagel states that the first epidemic of dengue fever occurred in the year 1779. At about the same time the disease was prevalent in Java and at Cairo and Alexandria. In the following year the affection was noted on the coasts of Coromandel, Arabia, and Persia. The same year it appeared in

Philadelphia and four years later, for the first time, it appeared in Europe (Cadiz and Seville). Epidemics were then noted in different parts of the world in 1818, 1824 to 1828, 1830 to 1870, and so on with unremitting frequency up to the present time. (See Siler, Hall, and Hitchens.)

The transmitting mosquito for this disease, *Aëdes ægypti,* was described by Linnæus in 1762, seventeen years before the disease was first noted in Java.

Distribution.—Dengue fever is most prevalent in tropical and subtropical countries. The disease has appeared as far north as Philadelphia and has been common in some of the Southern States.

The virus of dengue fever.—The virus of dengue fever is present in the circulating blood during the first three days of the disease. In 1903 Graham(3) reported the transmission of the dengue virus by mosquitoes. In this early work the exact species of mosquito responsible for the transmission of this virus was not definite. While Graham worked with *Culex fatigans* he was not certain that there were no *Stegomyia* (*Aëdes ægypti*) among his mosquitoes. In fact he admits that in perhaps all of his experiments both species were present. Subsequent work tends to confirm this. Graham also attempted to demonstrate the presence of the dengue virus in the mosquito by injecting a suspension of salivary gland, from presumably infected mosquitoes, under the skin of a volunteer. This patient developed a chill and high fever on the third day with an attack resembling dengue. In the blood Graham found a parasite which he thought to be a protozoan resembling the plasmodium of malaria.

In 1907 Ashburn and Craig(4) reported one case in nine persons bitten by *Culex fatigans.* All of these volunteers were soldiers who had passed through an epidemic of dengue at Fort William McKinley (Philippines) without having themselves been infected. Three of these cases were found to be immune; three possessed relative immunity (large amounts of infected blood only produced mild symptoms) ; one case may have had dengue before; in one case the incubation period was prolonged and another case was found to be susceptible to dengue upon subsequent infection although the mosquitoes refused to bite this patient during the experiment. In 1916 Cleveland, Bradley, and McDonald(5) in Australia infected four of seven persons with *Stegomyia fasciata* which had been transported to Sydney from an infected district. Experiments with

Culex fatigans were unsuccessful. The blood from infected cases when injected into susceptible volunteers reproduced the disease. This work in Australia was later confirmed by the work of Siler, Hall, and Hitchens in the Philippines. These authors experimented with sixty-four volunteers in the Philippines, and demonstrated further that the period of incubation in the mosquito ranges between eleven and fourteen days and that the virus is not transmitted hereditarily through the eggs of the mosquito.

The virus of dengue fever is filterable through Berkefeld filters according to the work of Ashburn and Craig. Its filterability has been confirmed repeatedly by subsequent investigators so that there no longer remains any doubt upon this point. While Cleland, Bradley, and McDonald were unsuccessful in some of their filtration experiments with this virus, they concluded that such failures of filtration were due to slow filtration and the plugging of the filter pores through which the fluid had to pass. Filtrates of blood from dengue-fever cases are capable of inducing the disease in susceptible individuals when injected intravenously. According to Koizumi, Yamaguchi, and Tonomura(6) the serum from infected blood which has been allowed to clot also contains the virus and is capable of inducing the infection in susceptible individuals. The virus remains viable in blood at ice-box temperature for over ninety hours but is readily destroyed at room temperature. Various organisms have been described as the causative agent in dengue fever, but no claims for etiology have as yet been substantiated. At present we may say that the virus of dengue still remains undetermined and uncultivated. While the epidemiology of dengue fever and yellow fever are quite similar, no direct evidence has been presented that the causative agents are similar. The possibility of the identity of the causative agents in these two diseases has been suggested, but there is no basis for such an assumption. According to proponents of this theory the dengue virus may represent a markedly attenuated or in some-way-changed form of the yellow-fever virus and eventually, so it is thought, when the true etiological agents in these two diseases have been determined, it will be found that they are at least somewhat similar in their nature. The recent work of Sellards,(7) which again raises doubt as to the significance of Noguchi's *Leptospira icteroides* in yellow fever, if confirmed, may again throw the etiology of yellow fever into the realm of the unknown, and, possibly, eventually with the filterable virus diseases. This should be encouraging for those who advocate the theory of the dual etiology of these two diseases. While speculation on this matter is permissible, any prophesy is entirely out of place. More recently Sellards and Siler have described rickettsiæ in mosquitoes (*Aëdes ægypti*) infected with the virus of dengue fever.

Incubation period in dengue.—Siler, Hall, and Hitchens found that "the average incubation period was six and one-twentieth days; in 25 per cent it was less than five days; in 60 per cent, less than six days; in 75 per cent, less than seven days; and in 90 per cent, less than eight days." These authors conclude that incubation periods of more than ten days must be very rare in the naturally acquired cases. Their results may be taken as confirming for the most part the work of previous investigators on this question.

Symptoms.—The onset is usually sudden without prodromal symptoms. Fever, headache, pain in the limbs and back, and a general feeling of malaise are usually the first symptoms noted. The appetite may or may not be impaired. Postorbital pain and tenderness are nearly always present. Mental depression is quite characteristic of the disease. Muscular and joint pains may be so severe as to make any movements of the body almost impossible. Children may have convulsions early in the course of the disease. There is usually deep congestion of the skin of the face and neck; the face appears swollen and the eyes suffused and congested. Erythematous mottling of the skin of the chest, back, arms, thighs, plantar and palmar surfaces, and the neck is usually present, but it may be entirely absent in some cases. The absence of such a rash should not rule out dengue fever. The temperature may rise rapidly to 105° F. or more and remain at a high level for twenty-four to forty-eight hours when usually there is a drop, then a rise within a day or two; sometimes a few days elapse before the second rise, forming a saddle-backed curve. A secondary skin rash usually appears when the temperature begins to return to normal. This rash is usually spoken of as "scarlatiniform" or "morbilliform" or "purpuric." It does not always appear, but when it does it is quite characteristic. Other symptoms such as jaundice, diarrhœa, loss in weight, epistaxis, glandular involvement, swelling of the joints, and photophobia may occur. Complications are rare. Death from dengue fever is unknown. The blood shows a tendency toward leucopenia, and in some cases the number of leucocytes may be markedly diminished. There is also a relative lymphocytosis.

Animals susceptible to the virus of dengue.—All experiments to infect animals other than man have failed. Efforts to infect hogs, dogs, guinea pigs, rats, rabbits, and monkeys have met with little success. Reports of the establishment of the disease in laboratory animals remain unconfirmed.

Immunity.—That a definite degree of immunity is produced by virtue of one attack of the disease appears to be established. However, this immunity is exceedingly variable. I have seen a patient the victim of this disease on two occasions within six months. In many individuals undoubted immunity is produced, which may last a year or longer. It seems quite apparent that some persons possess a natural immunity against this virus and reside in endemic and epidemic areas for many years without becoming victims of the disease.

Control measures.—Dengue fever, while never fatal, is important because of the great loss in time during the infection. Therefore, it becomes an important problem economically, and preventive measures should be instituted against it. The most important measure is the eradication of the transmitting mosquito. The incidence of the disease may be considerably lowered by the use of mosquito nets, particularly during the day time.

BIBLIOGRAPHY

1. SILER, HALL, and HITCHENS, Dengue: Its History, Epidemiology, Mechanism of Transmission, Etiology, Clinical Manifestations, Immunity, and Prevention, Bureau of Science Monograph 20 (1926) (lit.).
2. NOTHNAGEL, Encyclopedia of Practical Medicine (Am. ed.). Vol. on Malaria, Influenza and Dengue. W. B. Saunders & Co., Philadelphia and London (1905).
3. GRAHAM, Med. Rec. New York 61 (1902) 204–207; Journ. Trop. Med. London 6 (1903) 209–14.
4. ASHBURN and CRAIG, Journ. Infect. Dis. 4 (1907) 440–475; Philip. Journ. Sci. § B 2 (1907) 93–152; Journ. Am. Med. Assoc. 48 (1907) 692–693.
5. CLELAND, BRADLEY, and MCDONALD, Med. Journ. Australia 2 (1916) 179; Rep. Direct. Gen. Pub. Health N. S. Wales, Sydney (1916) 185–233; Journ. Hyg. 16 (1917) 317–420; 18 (1919) 217–254.
6. KOIZUMI, YAMAGUCHI, and TONOMURA, Trop. Dis. Bull. 12 (1918) 77–78; Abstracts Bact. 2 (1918) 240; China Med. Journ. 33 (1918) 355.
7. SELLARDS, Am. Journ. Trop. Med. 7 (1927) 71.
8. SELLARDS and SILER, Am. Journ. Trop. Med. 8 (1928) 299.

NAIROBI DISEASE OF SHEEP

Definition.—Nairobi disease of sheep is a specific, febrile, infectious disease of sheep and goats that is characterized by an acute hæmorrhagic gastroenteritis. The cause of the disease is thought to be a filterable virus that can be conveyed by the "brown" tick, *Rhipicephalus appendiculatus.*

History and distribution.—According to Montgomery(1) this disease was first noticed November 28, 1910, by Mr. H. Brassey Edwards, veterinary officer of the Nairobi district. Montgomery states that—

> sheep are in this country traded from the Masai, Suk, and Turkana countries and the Northern Frontier for disposal to the Kikuyu Native Reserves or to the Nairobi population. A very great number therefore pass through the Nairobi township annually, and the mortality among them while staying there is high.

For this reason the disease has been designated "Nairobi disease." The Kikuyu Native Reserve near Kabate has been naturally infected several times between 1910 and 1915. In 1911 Montgomery found this disease to be present in the Kedong Valley and at Juja near the Athi River. Montgomery further states that—

> in 1913 cases occurred at Makindu Railway Station, and in the same year many settlers on the Fort Hall Road purchased trade sheep which were passing from Fort Hall towards Nairobi. At purchase the animals appeared healthy, but within a fortnight nearly all died. Positive evidence of this disease was obtained from one farm.
>
> In 1914 the disease was diagnosed at Kikuyu Station; and it occurred at Voi, Burra, and Maktau among military slaughter sheep in this and the following year.
>
> The natives designate the disease "Kuharo" which means diarrhoea and about one half of the sheep born are lost. They have attributed its cause to diet. According to Montgomery he has never encountered any immune sheep coming direct from the Masai Reserve or from the Rift Valley and he believes that those districts are probably free from the infection. He states "as the direction of trade is away from these centers, it is probable that the disease has not yet had an opportunity of reaching them, and, in addition, it should be noted, the Masai Reserve and the Rift Valley are, in the main free from East Coast fever, a freedom probably due to a great paucity of the brown tick, the transmitter of both diseases.

The virus of Nairobi disease of sheep.—The virus of Nairobi disease is present in the blood stream of infected animals. It is also present in the serum of clotted blood, in saline extracts of organs, in the pericardial fluid, and in the urine. Montgomery failed to demonstrate its presence in bile. The virus may be present in the fæces of infected animals, but conclusive experimental proof of this is lacking. Infected blood after filtration through a Berkefeld candle remains infectious and produces the typical disease in test animals. The disease may be induced in susceptible animals following subcutaneous, intravenous, or intraperitoneal inoculation of infected blood. The typical disease may also be produced by intradermal inoculation such as the application of one or two drops of infected blood upon scarified areas of the ears. As little as 0.01 cubic centimeter of infected blood is sufficient to produce the disease in test animals, though 0.001 cubic centimeter failed to induce the infection. Susceptible animals may be infected with Nairobi disease per os, though simple contact, in paddocks free of ticks, does not lead to contraction of the disease. The adults of *Rhipicephalus appendiculatus*, which feed as nymphæ upon infected sheep, are capable of conveying the infection. Working with citrated or defibrinated blood or with plasma and serum, all from infected animals, Montgomery has shown in the following protocol that the virus of Nairobi disease is filterable through both Chamberland F and Berkefeld No. 7 filters.

TABLE 7.—*Nairobi disease.*

CHAMBERLAND F.

Virus.	Dilution.	Number inoculated.	Positive.	Negative.
Citrated blood	1:4	3	1	2
Do	1:9	5	4	1
Do	1:99	8	7	1
Do	1:999	1	1	0
Plasma	1:4	2	2	0
Do	1:9	8	7	1
Serum	1:9	11	9	2
BERKEFELD No. 7				
Citrated blood	1:4	2	1	1
Do	1:99	7	5	2
Plasma	1:4	2	2	0
Do	1:9	5	5	1
Serum	1:9	13	13	0
Do	1:99	5	4	1

From these experiments it is evident that the infectious agent in Nairobi disease should be classed with the group of ultraviruses.

An animal that has recovered from the disease does not harbor the virus. The virus in citrated blood or sterile serum remains viable for about twenty-eight days when exposed to the air and as long as forty-five days in sealed tubes. Virulent blood mixed with equal parts of oxalate-carbol-glycerol solution remains infective for about one week, which is illustrative of the marked resistance of this virus. The virus is destroyed by drying in storage for seventy-two hours at 37° C. The virus resists heating to 50° C. for two hours but is destroyed at 60° C. within five minutes.

Incubation period.—The incubation period varies from five to fifteen days in experimental cases of the disease. On the average by tick transmission it is about nine days.

Symptoms.—Within forty-eight hours following inoculation with infectious material in sheep, there is a rise in temperature to 105 or 106° F. The rise is sudden and remains between 105 and 108° F. for one or two days when it suddenly falls to normal. Within one or two days death or recovery results. Death usually occurs between four and eight days following inoculation with infectious material. In two hundred twenty-four sheep Montgomery found the average to be one hundred forty-eight hours. Following infection with ticks the incubation period averaged 9.4 days in Montgomery's experiments and the reaction 3.6 days. Upon the last day of the reaction (sharp fall in temperature) the infected animal appears dull and is somewhat off feed. The symptoms then become more marked. There is loss of appetite, marked depression, increased respiration and pulse, discharge of mucus from the nostrils, and an involuntary diarrhœa associated with much pain. The fæces are dark green. Coma may precede death by twenty-four hours. Diarrhœa is rare in cases that recover. The mortality varies according to breed of animals. Montgomery gives the following figures: Masai sheep, 71.5 per cent; Grade sheep, 31.5 per cent; Merino sheep, 30.7 per cent.

Animals susceptible to Nairobi disease.—Sheep (Masai, Grade, and Merino) are all susceptible. Grade Angora goats from the Rift Valley are susceptible to the infection. Both native and imported cattle, buffaloes, Arab and Indian horses, mules, donkeys, pigs, dogs, rats, mice, rabbits, and guinea pigs, all proved resistant to the virus.

Immunity.—One attack of the disease confers a definite protection against subsequent attacks. The exact length of time over which this immunity lasts is unknown, but it certainly lasts for several months and in some cases perhaps years. Different breeds of sheep show varying degrees of susceptibility. Grade and pure-bred show a much higher resistance than native sheep as judged from the mortality statistics. All attempts to devise methods for prophylactic immunization have failed. Antiserum preparations in sheep apparently possess marked hæmolytic qualities, a phenomenon that has also been noted in African horse sickness and remains unexplained.

Pathology.—Sheep dead of Nairobi disease exhibit typical anatomical changes in the gastrointestinal tract. The mucosa shows a variable degree of hyperæmia and œdema which is more marked in the large intestines. In folds it exhibits pronounced hæmorrhagic striæ. Usually the lumen of the intestines is empty but when containing fæces the mucosa is studded with petechiæ. The liver is only slightly enlarged, the gall bladder is distended, and the mucosa is frequently petechiated. The spleen is enlarged. The trachea and bronchi are hyperæmic and in some cases hæmorrhagic. The epicardium is petechiated, and the endocardium shows ecchymoses. The lymph glands are for the most part enlarged and congested.

Control measures.—The basis of prevention in this disease lies with the eradication of the tick. Dipping of cattle at three-day intervals has been suggested as a contributing measure to assist in the eradication of the tick. While it appears that the adult tick that in the nymphal stage fed upon infected animals, is capable of transmitting the disease, it has not been determined definitely if larvæ born of infected females can do so. In either case the eradication of the tick in all its stages of development is indicated.

Reference 1. MONTGOMERY, Journ. Comp. Path. and Therap. 30 (1917) 28–57.

CATARRHAL FEVER OF SHEEP

MALARIAL CATARRHAL FEVER, GEEL DIKKOP, BEKZIEKTE, OUIL BEK BLAW TONG

Definition.—Catarrhal fever of sheep is an acute febrile infectious disease which occurs in sheep in South Africa. It is characterized by hæmorrhagic stomatitis, marked œdematous swelling of the forehead and laryngeal region, and a cyanotic discoloration of the tongue.

History.—Catarrhal fever of sheep was first described by Hutcheson and Spreull(1) and later by Paine(2) and Theiler.(3)

For many years it has been prevalent in parts of South Africa and at times has caused great losses in sheep.

Distribution.—The disease is limited to South Africa and occurs in this region under local and periodical conditions similar to horse sickness.

Incubation period of catarrhal fever.—The incubation period on the average is about four days. In some cases it is slightly more or less.

Symptoms.—The disease is characterized by a rise in temperature to 42.5° C., dullness, and diminished appetite. A severe hæmorrhagic stomatitis develops accompanied by shedding of shreds of epithelium and a marked œdematous swelling of the forehead and the laryngeal regions. The tongue becomes swollen and blue. The swollen parts later may become hard and wrinkled; ulcerative keratitis and panophthalmitis, diarrhœa and icterus may develop. The mortality is about 40 per cent. In favorable cases the disease runs its course in about three weeks.

Animals susceptible to catarrhal fever.—The disease is infectious to sheep but not to goats. No other kind of animal is affected.

The virus of catarrhal fever of sheep.—The virus is present in the blood of infected animals during the course of the disease and is said to persist in the blood stream for nearly two months after recovery from the disease has taken place. Spreull transmitted the disease to sheep with filtered blood thereby demonstrating that it is one of the ultramicroscopic viruses. The natural infection appears to be transmitted by insects. The virus has never been cultured and little is known regarding its nature.

Immunity.—One attack of the disease protects animals from second attacks. Immunization of susceptible animals may be produced by simultaneous vaccination with 4 cubic centimeters of serum from highly immunized sheep and 2 cubic centimeters of virulent blood. A mixture of serum and virus, 2 : 1, is also said to give satisfactory results. Theiler states that in serial inoculation of sheep the virus becomes attenuated after about ten passages and then may be used for vaccine without fear of infection.

Pathology.—The anatomical changes include the local changes around the head, moderate swelling of the spleen, and signs of a generalized infection and anæmia.

Control measures.—Preventive measures consist in pasturing sheep on high dry pastures during the epidemic period of the year and in prophylactic immunization of susceptible animals.

BIBLIOGRAPHY

1. SPREULL, Journ. Comp. Path. 18 (1905) 321.
2. PAINE, Journ. Comp. Path. 19 (1906) 5.
3. THEILER, Schw. A. 37 (1895) 1; Bull. P. 3 (1905) 617; Z. f. Tm. 11 (1907) 301.

OTHER REFERENCE

HUTYRA and MAREK, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Eng. ed. by Mohler and Eichhorn. Alexander Eger, Chicago (1926).

AFRICAN HORSE SICKNESS

SÜDAFRIKANISCHE PFERDESTERBE (GERMAN); PESTE DU CHEVAL (FRENCH)
PAARDONZIEKTE, PERREZIEKTE (HOLLAND)

Definition.—African horse sickness, or "pestis equorum," is an infectious, noncontagious disease of horses and mules. It occurs in South Africa and is characterized by œdema of the subcutaneous and intramuscular tissues, catarrhal inflammation of the stomach and duodenum, and hæmorrhages in the internal organs. It is caused by a filterable virus.

History.—African horse sickness has been known for over one hundred years in South Africa. Prior to 1900 the disease was considered by Sander(1) to be a form of anthrax, and was confused by Rickmann(2) and Edington(3) with piroplasmosis. In 1900 McFadyean(4) proved that the virus of African horse sickness is a filter passer. This was soon confirmed by Nocard(5) in 1901 when he demonstrated that the virus passes through a porcelain filter. Important contribution has been made to the study of this disease by Theiler,(6) and a method of immunization has been devised by this author.

Distribution.—The chief distribution of this disease, as its name implies, is in South Africa. It has also been reported in Abyssinia, in East Africa, and in Arabia.

Incubation period.—The period of incubation in African horse sickness is usually six to seven days. (Theiler.)

Symptoms.—The disease occurs in two forms, acute and subacute. In the acute form, dunkop-paardenziekte, the temperature at first rises to 40 to 42° C. The appetite remains good, but toward the seventh or eighth day the animal becomes weak and the respiration is accelerated and labored. In most favorable cases the symptoms then gradually subside and the patient improves rapidly to recovery. In other cases œdema of the lungs develops and there is marked frothing from the mouth and nose. The lymph glands become swollen, and just before death the temperature drops; the animal dies from dyspnea and heart weakness.

The disease develops much more slowly in the subacute form, dikkop-paardenziekte. The symptoms in this form of the disease are much the same as in the acute form. Œdematous swellings appear around the orbits, and on the forehead, chest, and abdomen. The tongue may be markedly swollen, blue tongue, and there may be marked exophthalmia. In the majority of cases recovery takes place. The acute form of the disease is usually fatal, but the subacute form is much milder and most of the animals recover from this form of the disease. The disease is believed to be transmitted by some fly or other insect. According to some authors anopheles and stegomyia mosquitoes that have fed upon an infected horse within forty-eight hours serve to transmit the disease to healthy animals. Reinecke(8) produced a fatal disease in one horse by injecting an extract from ticks which he had collected in the African-horse-sickness district in Africa the year before.

Animals susceptible to the virus of African horse sickness.—The disease is limited to horses and mules. Donkeys do not become affected spontaneously.

The virus of African horse sickness.—Infected blood diluted with salt solution and filtered through Berkefeld or Chamberland B and F filters is capable of producing the disease in susceptible animals. Blood taken from horses during any stage of the disease produces the disease in susceptible horses when injected subcutaneously, intravenously, intratracheally, or in the lungs, or administered per os in doses ranging from 0.0005 to 1 cubic centimeter. The virus is also contained in the exudate of the bronchial secretions. The virus does not remain in the blood stream of animals that have recovered from the disease. According to Theiler the virus is quickly destroyed by the action of the sunlight and by drying, though it resists heating to 45° C. and 3 per cent phenol.

Horses are susceptible to artificial infection. Mules are less so. According to Edington it is possible to infect cattle fatally and to produce fever reactions in goats and sheep.

Immunity in African horse sickness.—One attack of the disease confers a mild immunity in that second attacks are never so severe as the first. It is said that an attack of the disease will protect about two-thirds of the animals from natural infection. Artificial immunity may be produced by a number of methods; the Theiler method is considered the best. This is a simultaneous method and consists of the injection of immune serum at the time that a small dose of virulent blood is given. Upon the appearance of a febrile reaction following the immunization a second injection of serum is administered. A small number of animals are lost by this method, but generally speaking it is most satisfactory. Still other methods of immunization have been advanced by Koch(7) and by Rickmann. In the preparation of the immune serum recommended by Theiler it is best to use a number of strains as it appears that the serum prepared from one strain only will not protect against all strains of the virus.

Pathology.—The chief findings at autopsy in animals dead of African horse sickness are infiltration of the subcutis and intramuscular tissue, acute swelling of the lymph glands, and catarrhal swelling of the mucous membrane of the stomach, hæmorrhages in the intestines, in the heart muscle under the endocardium, and in the kidneys. The liver and spleen may be hyperæmic and enlarged.

Control of African horse sickness.—Pasturing animals in high and noninfected pastures during the summer time is recommended, and it is thought that by breeding immune animals resistant breeds may in time be produced. Immunization by Theiler's method is recommended.

BIBLIOGRAPHY

1. SANDER, A. f. Tk. 21 (1896) 249.
2. RICKMANN, B. t. W. (1895) 289; A. f. Tk. 33 (1907) 372; (1908) 883.
3. EDINGTON, The Vet. 41 (1895) 595; Journ. Comp. Path. 13 (1900) 223, 281.
4. MCFADYEAN, Journ. Comp. Path. 13 (1900) 1; 14 (1901) 103.
5. NOCARD, Bull. 37 (1901).
6. THEILER, Schw. A. (1893) 145; D. t. W. (1901) 209; Bull. P. 3 (1905) 617; Rep. of the Gov. Vet. Bact. (1905–08).
7. KOCH, A. f. Tk. 31 (1905) 330.
8. REINECKE, Diss. Bern. (1909) (lit.).

OTHER REFERENCES

LEIPSIGER, Diss. Bern. (1909) (lit.).
FREI, Z. f. Infkr. 6 (1909) 363.

HOERAUF, Diss. Bern. (1910) (lit.).
BEVAN, V. J. (1911) 402.
KUHN, Centralbl. f. Bakt. (1911) 50, 31.

PAPPATACI FEVER

PHLEBOTOMUS FEVER; SAND-FLY FEVER; THREE-DAY FEVER

Definition.—Pappataci fever is an acute infectious disease characterized by its sudden onset and short duration (three days). It is probably due to a filterable virus.

History.—A disease characterized by fever and of three days' duration has been known to occur in the Mediterranean region for more than one hundred years. According to Vaughan(1) it was prevalent in Malta during the Crimean War and in the seventies of the last century was noted by Italian physicians and Austrian physicians stationed on the Adriatic coast. Doerr and Russ(2) in 1909 demonstrated the infectivity of the blood in this disease. Taussig (1905) and McCarrison (1906) called attention to the sand fly *Phlebotomus pappatassii* as the possible carrier of the disease. For this reason "phlebotomus fever" and "pappataci fever" have been employed to designate

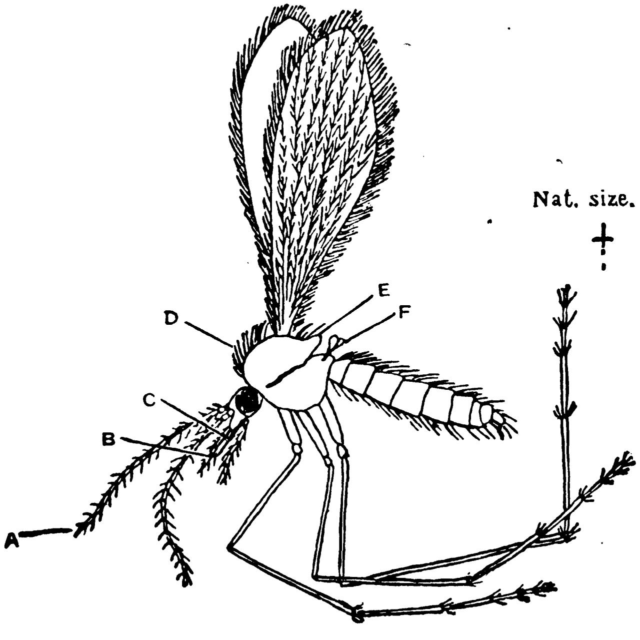


FIG. 2. Female phlebotomus, vector in pappataci fever; *a*, antenna; *b*, palps; *c*, probocis; *d*, prothorax; *e*, mesothorax; *f*, halteres. (After Alcock; from Manson.)

the disease. On account of its duration the disease is frequently referred to as "three-day fever." In 1913 Birt(3) published a review of this disease and cited the literature on the subject up to that time.

Distribution.—While the disease was first noted in the Mediterranean region, it has been reported as occurring in Switzerland, the Balkans, Greece, southern Russia, Egypt, Sudan, Uganda, China, India, South America, and the southern United States and California. Vaughan states that the phlebotomus has never been present in Bermuda and the disease has never been prevalent there.

The virus of pappataci fever.—The virus of pappataci fever is present in the blood stream of man during the first twenty-four hours of the disease. It is filterable through a Berkefeld filter as is the virus of dengue fever. In many of its clinical aspects the disease resembles dengue fever and influenza. The virus has not been cultivated and remains unidentified.

Incubation period.—The incubation period in this disease is usually given as three to seven days. While the prodromal symptoms are not marked there may be a general feeling of lassitude with slight aching pains in the back and limbs.

Symptoms.—The onset of the disease is sudden. Chills; fever; pains in the back, head, and limbs; congestion of the conjunctiva; irritability; and general lassitude are all characteristic of the disease. The temperature may rise to 102 or 105° F. In some cases there is a dry cough. The blood shows a marked leucopenia and a decrease in the percentage of polymorphonuclear cells. In some cases there may be a slight measleslike rash as in dengue fever. After two days the temperature begins to fall, and during this stage of the disease there is a tendency to nose bleed and vomiting. The patient quickly recovers, and there is no mortality. In July, 1918, several American Army divisions in France were affected by this disease, the course of the affection being typical of the description given above.

Animals susceptible to the virus of pappataci fever.—In so far as is known man is the only species susceptible to this virus.

Immunity.—It is said that one attack of this disease confers a definite immunity against subsequent attacks. (Rosenau's Preventive Medicine and Hygiene, 5th ed., p. 319.)

Control measures.—The phlebotomus is a small fly, gnat, or midge, about 3 millimeters in length. On account of its small size the usual preventive measures employed against biting insects such as sleeping under nets are of no avail. Destruction of breeding places and of the adult fly is to be recommended as perhaps the best fundamental measure for the control of this disease.

BIBLIOGRAPHY

1. VAUGHAN, Epidemiology and Public Health. C. V. Mosby Co., St. Louis **1** (1923).
2. DOERR and RUSS, Archiv f. Schiffs- u. Trop.-Hyg. **13** (1909) 693.
3. BIRT, Journ. Roy. Army Med. Corps **21** (1913) 389 (lit.).

NOTE

Since the section on yellow fever was prepared several reports have been published by the Yellow Fever Commission on the Gold Coast regarding their investigations of this disease. It has been demonstrated that the macacus monkey is susceptible to the virus of yellow fever, while the local African monkey, guinea pigs, Indian crown monkey, and chimpanzee are insusceptible. Further, it has been shown that convalescent serum will protect *Macacus rhesus* against the disease. The virus is not transmitted to the second generation of mosquitoes through the eggs, and apparently the virus is not filterable as it exists in the mosquito. It has also been shown by Bauer that *Aëdes ægypti* is not the only mosquito capable of transmitting the disease. Bauer and Hudson have also demonstrated that the yellow-fever virus may penetrate the unbroken skin and produce infection in monkeys. Further reports from this commission are awaited with anticipation.

CHAPTER VII

FILTERABLE VIRUS DISEASES OF ANIMALS

APHTHÆ EPIZOÖTICÆ: FOOT-AND-MOUTH DISEASE

FIÈVRE APHTHEUSE, COCOTTE (FRENCH); MAUL UND KLAUENSEUCHE (GERMAN); FEBRE AFTOSA (ITALIAN)

Definition.—Aphthous fever, or foot-and-mouth disease, is an acute contagious disease of cloven-footed animals which is characterized by a vesicular eruption on the mucous membranes and skin. It is caused by an ultramicroscopic virus.

History.—Foot-and-mouth disease was well known as early as 1764. During this early period it was thought to be caused by atmospheric and climatic conditions. There were those who suggested that it might even be caused by food poisoning. However, it is said that Sagar in Norway at this time recognized the fact that the disease is contagious. In fact it is well recognized now as perhaps the most contagious disease known to affect animals. In former times the disease spread with great rapidity and in the course of two years it had spread over the whole of Europe. Great epizoötics of the disease have come and gone. The last big outbreak occurred in Europe in 1887. This outbreak originated in Russia and soon invaded all of Europe and is still present. The disease was especially severe during the World War and great losses were suffered throughout Europe. In South Germany, for example, in 1920 there were 23,369 infected townships and 181,067 premises were affected. In France during this period there were 6,192 infected townships and over 37,000 premises affected. At the same time England suffered the loss of nearly 4,000 cattle, over 9,000 sheep, and 1,700 hogs from the ravages of this disease. Holland, Austria, and Hungary were also similarly affected. The disease has invaded the United States several times during the last half century. In 1914 and 1915 the disease was of very serious importance to the stock industry in America, but it was soon brought under control.

Distribution.—Foot-and-mouth disease in its distribution is practically world wide. It is present or has been present in North and South America, Europe, Asia, and Africa.

The virus of foot-and-mouth disease.—Prior to 1890 aphthous fever was thought to be caused by bacteria or protozoans. Various bacterial and protozoan forms had been described. In 1897 Loeffler and Frosch(1) proved that the virus of foot-and-mouth disease will pass through the pores of a Chamberland or Berkefeld filter. This observation was later confirmed by Hecker(2) and by Nocard(3) and since 1900 has been repeatedly confirmed by other investigators. The virus is present in the contents of the vesicles and during the early stages of the disease can be demonstrated in the blood stream. The milk may contain the virus before any vesicles appear and often remains infective during the entire febrile period of the disease. Other secretions of the body, such as the saliva, tears, and nasal discharge, may contain the virus as a result of contamination with vesicular contents. The vesicular contents may include various bacteria, such as streptococci, staphylococci, and sarcinæ. These bacteria may be regarded as secondary invaders and of no etiological significance, although they may influence the course of the disease and later produce deep ulcers, mastitis, inflammation of the hoofs, and pyæmia. Frosch and Dahmen(4) recently reported the cultivation of the virus of foot-and-mouth disease by a method based primarily upon the concentration or sedimentation of the virus by centrifugation at 3,000 revolutions per minute for one-half to one and a half hours. The British Committee,(5) Abe,(6) Gins,(7) and Olitsky and Boëz(8) have all been unable to confirm the work of Frosch and Dahmen. Olitsky and Boëz have shown that after two hours centrifugation of the virus in active guinea-pig blood, aspirated lymph, or suspensions of ground infected pad tissue, at 2,500 to 3,000 revolutions per minute, the topmost portion of the specimen was as active as the lowest layer. These authors point out that the inability to sediment the virus may indicate that the active agent is very minute, but not necessarily that it is of the nature of a contagium vivum fluidum. These authors were able to infect guinea pigs and propagate the virus of foot-and-mouth disease through two hundred sixty-one passages in this animal. The virus is markedly epitheliotropic. They state that over two thousand animals proved susceptible to the virus, and cattle and hogs could again be infected without any difficulty with the virus propagated in guinea pigs. They observed no natural immunity in guinea pigs to the virus. Their virus, obtained originally from an infected cow, was active in dilutions of 1 : 10,000,000 and they concluded that "the rate and energy of action of the virus were proportional to its concentration, thus differing from the behavior of certain enzymes." They believe that the failure of deposition of the virus by centrifugation is related to the minute size of the infectious agent. They found at least two types of virus corresponding to Vallée's types "O" and "A." These do not cross-immunize.

In a further report Olitsky and Boëz have demonstrated by catarphroresis experiments that the virus of foot-and-mouth disease carries an electro-positive charge. Its isoelectric range is at about P_H 8. This fact is exceedingly interesting and pertinent to the study of filterable viruses for it indicates that the viruses of this group are different from ordinary bacteria in respect to their electric charge; it indicates the possible separation of the virus from protein and suggests an explanation of the high resistance of viruses of this nature to certain chemicals. These authors also succeeded in filtering the virus of foot-and-mouth disease through Chamberland, Berkefeld, and Seitz filters as well as through collodion membranes and Bechhold's ultrafilter. Filtration through collodion membranes was regarded as successful only when the thinnest membranes were employed and in general the method was not considered satisfactory. With the Bechhold filter Olitsky and Boëz determined the relative size of the virus of foot-and-mouth disease to range between 20 and 100 millimicrons in diameter. These results conform closely to those of Zinsser and Fei-Fang Tang(9) in later experiments showing an order of various substances as follows: Crystallized egg albumen; crystallized serum albumin; trypsin; collargol, casein; bacteriophage, Rous sarcoma, and herpes virus; and arsenic trisulphide.

In 1908 Terni(10) observed a protozoan (*Cytorhyctes*) that measured about 0.5 micron in the vesicular lymph and in the internal organs of over four hundred cattle affected with foot-and-mouth disease. Later, in 1911–12, Siegel(11) demonstrated cocci (*Cytorhyctescoccus*) whose diameter measured 0.1 to 0.2 micron in the blood, vesicular lymph, cell plasma, and heart muscle of affected animals. Huntemüller(12) described coccus-like globules in the vesicular lymph and in the epithelial cells that he thought were the causative agents in the disease. However, it seems altogether likely that Huntemüller's globules were in reality fat droplets. Betegh(13) described in 1911 very small motile bodies, 0.25 to 0.1 micron, in the epithelial cells of the vesicles and later free in the lymph. Such bodies as these, however, have been observed in normal tissue fluids under dark field and little significance is attached to their presence.

In spite of all the data that have accumulated purporting to identify the etiological agent of foot-and-mouth disease with bacterial and protozoan forms that are visible under ordinary magnification, we must, in the light of modern investigation, conclude that the infective agent in this disease is ultramicroscopic and filterable.

The virus of foot-and-mouth disease is destroyed in about twenty-four hours, by drying at room temperature. Diluted virus hermetically sealed in tubes will retain its virulence for three or four months. It is destroyed at 50° C. within twenty minutes. It resists 70° C. for nearly ten minutes, while 100° C. destroys it immediately. In milk the virus is destroyed during the process of souring.

Infections result experimentally in cattle following the intravenous administration of 0.005 cubic centimeter of fresh pure lymph. Artificial infection may also be effected by rubbing virulent lymph into the scarified skin.

Incubation period in foot-and-mouth disease.—In artificial infections, depending upon the amount and virulence of lymph injected, there is an incubation period of one to six days. The first lesions appear on the mucous membrane of the mouth, and within one or two days later lesions appear upon the feet. In the natural infection the incubation period ranges from two to seven days.

Symptoms.—Natural infection may occur directly when healthy animals come in contact with infected animals. Presumably the virus enters the susceptible host by way of the mucous membranes. Indirect transmission is far commoner, however, than direct spread of the disease. The body excretions and secretions of affected animals contaminate stables, yards, cribs, food, drinking water, pastures, highways, and all places to which infected animals have access. Attendants may also transmit the disease from infected animals to healthy animals.

Following intravenous injection in cattle, the disease begins with a rise in temperature during the first two days up to 40 or 41° C. As soon as the vesicles begin to appear the fever starts to descend and the disease runs its course without fever. Along with the initial fever there is a rapid pulse and diminished appetite.

While lesions in the mouth are commoner in cattle than in sheep, goats, and hogs, the buccal mucous membrane becomes sensitive and the animal may cease to eat entirely. The mucous membrane of the lips and gums is dry and reddened. Saliva accumulates in great quantities and falls from the mouth when it is opened. Usually on the second or third day after onset of symptoms vesicles appear which at first contain a clear watery fluid that later becomes grayish white. After one or two days the vesicles break and a painful flat erosion remains. These erosions soon become covered with new epithelium and the animal again begins to eat. In cattle vesicles also develop on the muzzle, and at the base of the horns, and in hogs upon the snout. Vesicles may in some cases also appear in the nasal passages and upon the conjunctiva. In other cases the

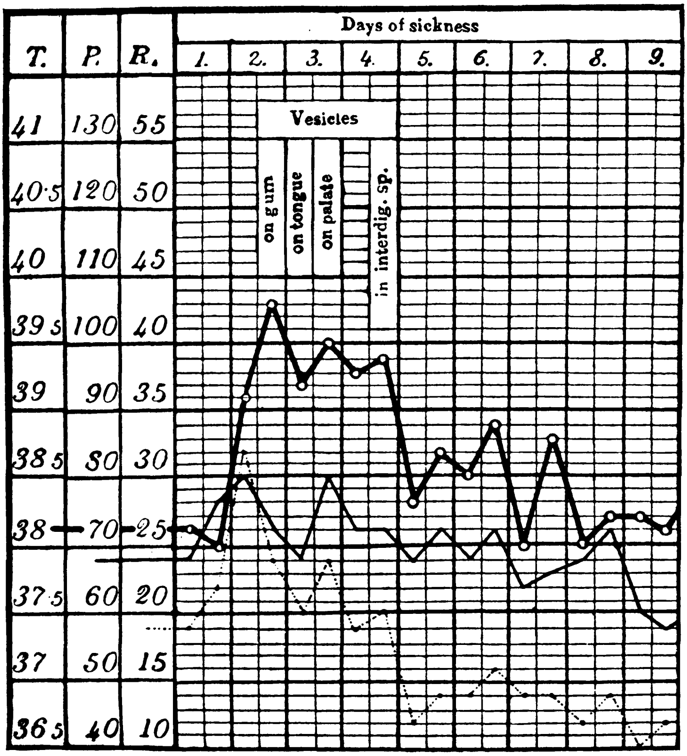


FIG. 3. Fever curve in a typical case of foot-and-mouth disease. (From Hutyra and Marek.)

pharynx may also become involved and the affection may spread to the trachea and lungs.

The hoof infection usually develops at the same time or directly following the eruption on the buccal mucous membranes. At first the animal shows signs of lameness and the affection may be so severe that the animal refuses to walk at all and lies on the ground. Vesicles appear on the skin of the coronary band, the heel of the foot, and the interdigital space. These vesicles usually burst before those in the mouth and become mixed with dirt and form crusts. New epithelium is gradually formed, and healing with scab formation takes place. In some cases the coronary band becomes separated from the horny border and a crack may remain between the newly formed horn and the old wall and healing may require several weeks. In cattle the vesicular lesions frequently appear upon the udders. These lesions may become serious and lead to painful swelling of the udder and catarrh of the milk duct.

Complications such as spread of the lesions to the trachea and lungs, formation of ulcers, septicæmia, and pyæmia as a result of exogenous bacterial invasion, etc., may occur. In goats and sheep the affection is usually mild, though severe outbreaks have occurred. In some cases the symptoms may be so mild that the affection remains unnoticed. In hogs the extremities are chiefly affected and more rarely the mucous membranes. In cattle abortion frequently results. In young stock the losses from foot-and-mouth disease may be from 40 to 60 per cent of the newly born or very young calves. This was true in the Hungarian epidemic in 1910. Malignant forms of the disease have been reported in which, without complications, the disease usually results in death of the cattle. Such forms of the disease were evidenced immediately following the World War. However, in general, the great majority of cases of foot-and-mouth disease run a favorable course. In uncomplicated cases the disease runs its course in two to three weeks. While some cases are left permanently affected with chronic disturbances of the hoof, the majority recover completely.

Animals susceptible to the virus of foot-and-mouth disease.— Cattle, hogs, sheep, and goats are apparently susceptible to this virus in the order given. Other susceptible animals include buffaloes, reindeer, camels, and exceptionally horses, dogs, and cats. Guinea pigs and rabbits have been used for experimental purposes. Mild infection with foot-and-mouth disease virus may occur in man and is charecterized by a gastrointestinal catarrh.

Immunity in foot-and-mouth disease.—One attack of the disease confers a definite immunity to second attacks. This immunity is said to last for about one year. Cases have been known to become reinfected within six to ten weeks and even after ten to twelve days. Calves from cows that have recovered from the disease at an advanced stage of pregnancy, according to Loeffler and Frosch, may be immune from both natural and artificial infection. Feeding of milk from an immune cow does not produce any immunity. According to Möbius(14) the virus may be transmitted from mother to calf and the young calf may be born with the disease.

While many methods of immunization have been advocated for the prevention of foot-and-mouth disease, it may be stated that at present there is no method satisfactory for general use.

Pathology.—Autopsies reveal several characteristic changes as a result of the infection, such as the following: Vesicular exanthema, acute catarrhal swelling of the mucous membranes of the respiratory tract, minute hæmorrhages on serous membranes such as the visceral layer of the pericardium, in some cases ulcerations on the mucous membranes of the respiratory tract and intestinal tract, œdema of the valves of the heart, enlargement of the spleen, fluid in the pericardial sac, and in cases of complication evidence of septicæmia and pyæmia may be noted. The changes in the heart muscle were first described by Johne(15) and consist in hyaline degeneration of the muscle fibers, and in prolonged cases evidence of necrosis and of connective-tissue regeneration may be noted. These changes have been thought to represent a myxolytic process induced by specific foot-and-mouth disease toxin.

Control measures in foot-and-mouth disease.—While the loss of milk and loss of working hours are the chief economic factors in this disease, it has been pointed out that in certain epidemics the mortality of the disease may be exceedingly high. Prevention and control consists in isolation of infected animals and quarantine of new animals that are to be introduced into old herds. Calves should be prevented from sucking their mothers and should receive milk that has been heated to 70° C.

BIBLIOGRAPHY

1. LOEFFLER and FROSCH, Cbl. f. Bakt. 22 (1897) 257.
LOEFFLER, D. t. W. (1899) 317; B. m. W. (1803) 685; Kongr. Haag. (1909).

2. Hecker, B. t. W. (1898) 61; (1899) 6, 130.
3. Nocard, Rev. gén. **1** (1903) 369; Acad. de méd. (1901) Arb. z. Erforschung d. M. u. Kls., Denkschr. d. kais. Ges-Amtes. (1901).
4. Frosch and Dahmen, Berl. tierärztl. Woch. **40** (1924) 185, 273, 341; Arch. wissensch. u. prakt. Thierheilk **51** (1924) 99.
5. British Committee, First Progress Report of the Food-and-Mouth Research Committee, Ministry of Agriculture and Fisheries, London (1925).
   Arkwright, Burbury, Bedson, and Maitland, Journ. Comp. Path. and Therap. **38** (1925) 229.
   Stockman and Minet, Journ. Comp. Path. and Therap. **39** (1926) 1.
6. Abe, Z. Infektionskrankh. Haustiere **28** (1925) 111.
7. Gins, Berl. tierärztl. Woch. **40** (1924) 661.
8. Olitsky and Boëz, Journ. Exp. Med. **45** (1927) 673–683; 685-699.
   Olitsky, J. A. V. M. A., **70**; N. S. **23** (1927) 926.
9. Zinsser and Fei-Fang Tang, Journ. Exp. Med. **46** (1927) 357.
10. Terni, D. t. W. (1908) 747.
11. Siegel, B. t. W. (1911) 909; (1912) 189, 713.
12. Huntemüller, Ebenda **61** (1912) 375.
13. Betegh, Cbl. f. Bakt. **60** (1911) 86.
14. Möbius, S. B. (1895) 75.
15. Johne, S. B. (1881) 62; D. Z. f. Tm. (1884) 186.

OTHER REFERENCES

Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Eng. ed. by Mohler and Eichhorn. Alexander Eger, Chicago (1926).

Kelser, Manual of Vet. Bacteriology. Williams and Wilkins, Baltimore (1927).

Hertwig, Mag. **8** (1842) 389.

Haubner, Vet. Police (1869) 359 (lit.).

Siedamgrotzky, S. B. **71** (1892).

Hess, Klauenkrankh d. Rindes (1892).

Schütz, A. f. Tk. **20** (1894) 1.

Mohler and Washburn, Bureau of Animal Industry Bull. **63** (1905).

Casper, B. t. W. **399** (1907) (lit.).

Kronacher, Z. f. Tm. **16** (1912) 49.

Schäffer. A. f. Tk. **20** (1894) 331.

Bräuer, S. B. (1876) 84.

Woestendieck, Pr. M. (1883) 9.

De Jong, D. t. W. (1911) 680.

Korányi, Nothnagels Handvuch, V. Bd. I. T. (1900).

HOG CHOLERA: SWINE FEVER

TYPHOID FEVER; CHOLERA SUUM, PESTE DU PORC (FRENCH); SCHWEINEPEST (GERMAN); PESTE PORCINA (ITALIAN)

Definition.—Hog cholera is an acute septicæmic disease of swine caused by a filterable virus and characterized by an acute febrile reaction with inflammatory swelling of the conjunctiva, eczematous eruption of the skin, followed by diarrhœa.

History.—In 1885 Salmon and Smith first described this disease as an affection of hogs in America. These authors designated a short motile rod, *Salmonella suipestifer,* as the cause of this disease and further differentiated this condition from swine septicæmia, or swine plague, which was recognized in Germany about the same time. However, the American authors demonstrated that the two affections often appeared at the same time in the same animal and attributed the severity of the disease in American hogs to the dual infection. This interpretation was concurred in by other investigators of the time, notably Moore and de Schweinitz in America and by Raccuglia and Affanasieff in Europe. However, the frequency of the dual or mixed infection caused certain investigators in America and in Europe to question the identity of the two diseases though no experimental proof was available at that time to substantiate their doubts. *Bacillus suipestifer* had been found in the blood, the liver, and the spleen; this organism was known to produce a toxin in culture media; attenuated cultures had been used as vaccines and conferred a certain immunity, and inoculation of cultures produced a disease with symptoms and lesions similar to the natural disease. Now it is known that the disease produced by artificial inoculation is not contagious, while the natural disease is highly contagious.

It was not until 1904 that de Schweinitz and Dorset(1) reported a disease occurring in the state of Iowa which clinically resembled hog cholera and found that this disease could be transmitted from diseased animals to healthy animals by filtered bacteria-free blood. Later Dorset, Bolton, and McBryde(2) proved that this disease was in reality identical with hog cholera and advanced the idea that *Bacillus suipestifer* was merely an associated organism and produced lesions only secondarily in the already infected animal. This work was quickly confirmed in America by Clintock, Boxmeyer, and Siffer(3) and in Europe by Hutyra(4) and Ostertag;(5) Theiler(6) in South Africa reported similar findings. Thus the etiology of hog cholera was established as due to a filterable virus and methods of treatment and immunization were directed into new channels. According to Joest there is no longer any reason for distinguishing between "hog cholera in the narrow sense" (pure hog cholera and mixed infection) and "hog cholera in a broader sense" (the two forms of "bacillary hog cholera"). Furthermore, according to Hutyra and Marek such distinction is not justifiable, for the reason that the last-named disease is not hog cholera. (Paratyphoid in pigs.)

Distribution.—It is said that hog cholera first appeared in the state of Ohio in 1833. From Ohio it spread over the entire United States. It was first established, according to all available records, in England in 1862. In England in 1896 it caused a lose of 30 per cent of all hogs in the country. It is supposed to have spread from England to Sweden in 1887. The same year it appeared in Denmark and in Amagar Island. About the same time the disease appeared in France, Spain, and Italy. There was a serious outbreak of hog cholera in Germany in 1893 and from there it is thought to have spread to Prussian Silesia, Austria, Hungary, Russia, and Roumania in 1895.

It is estimated that in the United States this disease caused losses in 1873 of twenty million dollars; in 1882, thirteen millions; in 1883 and 1885, twenty-five and thirty millions, respectively, while in 1888 the loss was estimated to have affected 45,000,000 hogs of a value of 200,000,000 dollars. In 1903 Salmon estimated the loss due to this disease at 50,000,000 dollars, and Kinsley in 1914 estimated the loss at 100,000,000 dollars. In 1918 in the United States out of a total of 75,000,000 hogs, Mohler estimated that 2,815,900 became victims of hog cholera, The disease is also known in Central and South Africa where it causes great losses.

Incubation period.—The incubation period of hog cholera in artificial infections produced by breeding healthy with infected hogs is at least four days and usually thirteen to eighteen days. When the disease is produced by injecting virulent blood subcutaneously the incubation period is from eight to ten days, exceptionally five days and in some cases as long as two to three weeks.

Symptoms.—The first symptom is an elevation in temperature. Other symptoms may not appear for two or three days after the onset of fever. In the severe cases the temperature remains almost at a constant level until death. In less-severe cases it may drop and in favorable cases return to normal.

Following the rise in temperature the first symptom noticed is the lack of appetite. An acute conjunctivitis with mucous or mucopurulent secretion is present early in the disease. Vomiting of the stomach contents stained with bile may appear very early. There is at first constipation but diarrhœa shortly sets in. The animal becomes weakened, and if improvement does not

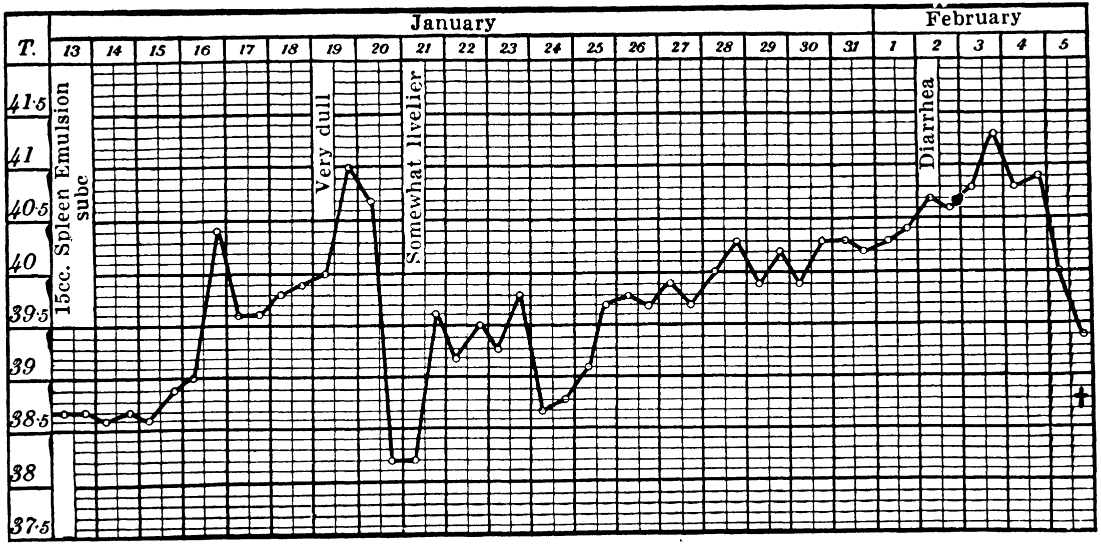


FIG. 4. Artificial hog-cholera infection with filtered material from a hog affected with cholera. The first rise in temperature is caused by the primary infection, the second by the secondary infection. (After Hutyra and Marek.)

ensue the animal dies within four to seven days with septicæmia.

The mixed form is less acute though inflammatory changes appear on the buccal mucous membranes and may be observed by exploring the buccal cavity. Hæmorrhages from the body cavities are not rare, and pneumonias are frequent complications which contribute to the high mortality of the disease. Where only the intestinal symptoms are manifested the prognosis in the disease is much better.

Animals susceptible to hog cholera.—Hogs of all breeds and of all ages are susceptible to hog cholera. Higher-bred hogs and younger animals are more susceptible than others. In infected localities where the older animals have had the disease the disease is limited to the younger growing animals. Pigs of diseased sows if they become affected usually die. Usually pigs of immune sows resist artificial infection but may become infected on infected premises. After birth, particularly after weaning, young pigs lose their immunity and the immune bodies that are present in the milk vanish very rapidly.

The virus.—In the infected animal the virus of hog cholera is present in the blood, bile, fæces, urine, conjunctival secretions, and in all organs. The virus is filterable through Berkefeld filters.

All attempts to demonstrate the virus under the highest magnification or to cultivate it in artificial culture media have failed. Unlenhuth has described certain cellular inclosures in smears prepared from the conjunctiva. These inclosures represent very fine granules similar to the so-called trachoma bodies in man to which reference is made in Chapter I. Such inclusion bodies are considered by Prowazek and Halberstädter as parasites.

Hutyra(7) states that the virus adheres to the red blood cells and is not separated from the red blood cells after repeated washings. In fact the virus has been found in the serum which has separated from the blood clots, and according to Meyer the virus is absorbed by the red blood cells of other species. Ruther found a very small spirochæte, *Vibrio suis,* in the ulcers of the intestines and in lymph glands and ulcers which appeared upon the skin. Such organisms have also been demonstrated in the blood stream of infected hogs. Drake found similar spirochætes in American hogs and named them *Spirochete hyos* and such spirochætes are said to pass through Berkefeld filters in the form of fine granules. Arnheim regards these spirochætes only as saprophytes. Healy and Gott observed small round and elongated bodies which, after staining by Giemsa's method, measured about 0.2 to 0.3 inch in size. These bodies were apparently inclosed in a gelatinous substance, but their significance is doubtful.

Two bacterial forms have been associated with hog cholera. One is known as *Bacillus suipestifer;* the other, which is known as *Bacillus suisepticus,* is identical with the organism of pure swine plague.

Bacillus suipestifer (*B. cholera suis*) belongs to the paratyphosus B-group. It resembles the colon bacillus and occurs chiefly in pairs or singly, but in cultures it forms chains and filaments. It is motile and possesses flagella arranged peritrichally. It is Gram negative and grows either aërobically or anaërobically at room temperature or at body temperature. On agar bluish, flat, transparent, round colonies appear, and on gelatin the colonies are quite similar. Gelatin is not liquified. Dextrose is fermented but lactose is not, thus differentiating it from *B. coli.* Mice, guinea pigs, and rabbits are highly susceptible to this organism, and pigeons are only slightly susceptible. A disease characterized by inflammation and ulceration of the intestines and lymph glands as well as a hæmorrhagic septicæmia is produced in hogs when cultures of this organism are fed, injected subcutaneously, or injected intravenously.

Immunity in hog cholera.—One attack of the disease confers a definite immunity. We have seen that young pigs possess immunity following birth if the mother sow has passed through the disease. This immunity, however, is only temporary.

Practical immunization has been carried on in a number of ways. In Hungary immunization with blood serum from slaughtered or recovered animals has been used, but the results were not very promising. Passive immunization with an immune serum prepared in hogs which had acquired an immunity from natural or artificial infection, by injecting virulent blood, has given fairly satisfactory results in that such a serum protects susceptible animals for about three weeks. Such a serum also appears to have some curative value especially during the incubation period of the disease. Such injections may be given at later periods to prolong the immunity.

The most effective method of immunization in hog cholera is known as the simultaneous method. According to this practice susceptible animals are given subcutaneous injections of about 20 cubic centimeters of immune serum and at the same time 1 to 2 cubic centimeters of virulent blood. Immunity conferred in this manner lasts about six months, though the method is not without danger in that some of the animals become symptomless carriers and are therefore a source of danger to susceptible animals. It is thought that one reason for this is the varying virulence of the virus and the fact that there is no way at present of standardizing the immune serum.

All attempts to prepare an efficient vaccine from the tissues of infected animals have failed. Likewise the preparation of a vaccine from virulent blood by special methods of treatment, such as heating, chemical treatment, etc., has resulted in a vaccine of very doubtful value.

Pathology in hog cholera.—In pure hog cholera we are dealing with a hæmorrhagic septicæmia. The intestinal wall presents numerous small red hæmorrhages. Similar lesions are found in the pleura and epicardium though they also appear elsewhere in the body. They are frequently found in the skin, and the lungs may present the same picture or various-sized hæmorrhagic infarcts. Large hæmorrhages may be found in the endocardium and more rarely in the meninges. All lymph glands are swollen and their cut surfaces present numerous small hæmorrhages. The bone marrow is dark blackish red and is very characteristic.

In the mixed form of hog cholera there are definite changes that indicate secondary infection. The lymph follicles of the intestines contain hard caseous nodules, and deep ulceration is frequently present. In some cases the caseous nodules are also present in organs such as the liver, kidneys, spleen, lungs, udder, and bone marrow. Other changes, such as hæmorrhages in the skin and organs, necrosis of the gall bladder, urinary bladder, and vagina, are not uncommon.

Control measures in hog cholera.—Effective control measures that have been used in various countries include isolation of infected animals, reporting of the disease, quarantine measures, slaughtering of affected animals and destruction of carcasses, and immunization.

Hog cholera is definitely contagious and the Bureau of Animal Industry in the United States has conducted control in this disease since 1914 with increasingly better results. In their control measures vaccination is very important, and the Bureau controls the manufacture of biologic products for this disease according to government regulation.

BIBLIOGRAPHY

1. De Schweinitz and Dorset, Bureau of Animal Industry 20 (1903) Rep., 157.
2. Dorset, Bolton, and McBryde, Bureau of Animal Industry 21 (1904) Rep., 138.
3. Clintock, Boxmyer, and Siffer, Journ. of Diseases 2 (1905) 351.
4. Hutyra, B. t. W. (1906) 607.
5. Ostertag, B. t. W. (1906) 623.
6. Theiler, F. d. Vhyg. 4 (1906) 121.
7. Hutyra, Pathology and Therapeutics of the Diseases of Domestic Animals, Hutyra and Marek, 3d Am. ed. by Mohler and Eichhorn 1: 326.

RINDERPEST: CATTLE PLAGUE

TYPHUS BOVUM CONTAGIOSUS, PESTE BOVINE; TYPHUS CONTAGIEUX (FRENCH); ORIENTALISCHE RINDERPEST (GERMAN); PESTE BOVILLA (ITALIAN)

Definition.—Rinderpest, or cattle plague, is an acute, febrile, contagious, and infectious disease of cattle. It is characterized by fever, a fine pseudomembrane formation upon the mucous membranes, increased salivation, and in most cases a nasal discharge and profuse bloody diarrhœa.

History.—Rinderpest has been known since earliest times and is thought to have originated in Asia. Its infectious nature was recognized as early as 1744. In olden times it was considered identical with such diseases as smallpox, typhus fever, and dysentery in man. At one time it was thought to have a spontaneous origin, and its etiology remained a mystery until 1902 when Nicolle and Adil-Bey(1) demonstrated that the causative agent of this disease is filterable through porcelain filters. Since that time it has been classified definitely with the filterable virus diseases.

Distribution.—Rinderpest has always been present in Asia. In 1711 the disease was prevalent throughout Europe and caused enormous losses of cattle. During the Napoleonic Wars it is said to have caused great losses in Germany and France. During the Franco-Prussian War the plague again extended over middle Europe, but since 1881 it has been completely suppressed. Since that time it has occurred in Russia and Turkey, and during the World War the disease spread into Bulgaria and later into Roumania though it was quickly eradicated. In 1920 the disease was introduced from Asia into Latvia and on into Poland. It is said still to exist in northeastern Poland, though it has been eradicated elsewhere. During the same year the disease was introduced into Belgium with Zebu cattle from India and rapidly spread throughout Belgium so that eighty-five townships were involved. It was completely eradicated within a short time. Since 1921 cattle plague has been prevalent in various parts of Asia and Africa and is the most important disease of cattle in the Philippine Islands at the present time. The disease does not exist in the United States.

The virus of rinderpest.—Rinderpest virus is passed from animal to animal, chiefly through contaminated food and water. The virus is present in the blood stream of affected animals, in various tissues of the body such as brain, lymph glands, spleen, liver, heart, thymus, intestines, muscles, larynx, pharynx, and the base of the tongue. It is also present in the intestinal contents, cerebrospinal fluid, and peritoneal exudate. Berkefeld or Chamberland filtrates of the last three materials were demonstrated by Nocard(2) to be infectious. According to Koch,(3) Kolle,(4) and Theiler(5) blood serum from infected blood does not contain the virus. Theiler and Baldrey(6) believe that the virus is closely adherent to the blood cells, while Nicolle and Adil-Bey believed the virus to be inclosed in the leucocytes.

Nothing is known regarding the morphology of the virus of cattle plague. Braddon(7) thought that minute, punctiform, needle-shaped bodies within and adherent to the blood cells, as well as in the plasma, were the causative agents of this disease. These were considered by other investigators to be artifacts. Cultivation of the virus has not been successful. Boynton(8) states that the virus remains active in clotted blood longer than in defibrinated or citrated blood. In his experiments he has demonstrated that the virus remains virulent for sixteen days in fertile hens' eggs but not so long in nonfertile eggs. He further showed that virulent blood in open tubes at 40° C. becomes inactive after three days, which indicates that anaërobic conditions are necessary. This author has also demonstrated that 0.000337 cubic centimeter of infected whole blood is capable of transmitting the disease to suceptible animals while 0.00011 cubic centimeter and 0.0001 cubic centimeter failed to transmit the disease. Ten cubic centimeters of blood from infected animals was centrifuged at 3,000 revolutions per minute for three hours and it was found that the upper 7 cubic centimeters remained noninfective while the lower 3 cubic centimeters produced the disease. In two hours time the upper 7 cubic centimeters were also infective. Certainly the possible effect of heat generated during prolonged centrifugation such as Boynton has used should be taken into consideration in evaluating experiments of this kind. The virus at the top of the tube may have been destroyed by heat.

The virus of cattle plague is extremely sensitive to environment outside the body. Réfik-Bey (9) states that the "virus is essentially fragile and incapable of development in external media." Edington (10) has shown that the nasal mucus from spontaneous cases was found to lose its virulence very quickly when exposed to the air longer than twenty-four hours. Stockman (11) states that the virulent material of cattle plague does not remain active for more than a day or two outside the body. According to Yersin (12) two days of desiccation are sufficient to destroy the virulence of the blood. In contrast to these observations Ruediger (13) states that pastures which have been occupied by sick animals may remain infected for months or even years. According to Boynton's experiments the virus of cattle plague did not survive beyond twenty-four hours in corrals bare of vegetation but containing water. This author made his tests during various seasons of the year and found no variations in this respect. He found that susceptible animals placed in infected corrals became infected within half an hour, twelve hours, and seventeen hours. Further, he demonstrated that urine from infected animals contains the virus and when sprinkled on the grass remained infective for thirty-six hours. Fæces diluted with water and sprinkled on the grass remained infective for twenty-four hours.

Direct sunlight destroys the virus of cattle plague within two hours, and putrefaction is said to destroy it within a very short time. Two per cent phenol, 1 : 1000 corrosive sublimate, and 1 per cent milk of lime are all efficient virucides for this agent. The virus is said to exist in the blood and secretions of animals for about thirty days after recovery from the disease, but as long a period as one hundred forty days in which the virus remained in the lesions is on record. Some authors believe that the virus might be permanently eliminated by animal carriers. Cattle, sheep, goats, and hogs may carry the virus and not be effected by the disease, but this concept has not had definite proof. Certainly the ease with which some epidemics of the disease have been eradicated indicates that transmission of the disease by carriers is not such an important factor.

Incubation period in rinderpest.—The time of incubation in cattle ranges from three to nine days. In some cases the first symptoms of the disease may occur as early as twenty-four to thirty-six hours, while in exceptional cases the incubation period may extend from sixteen to twenty-four days. In 1867 the international veterinary congress met in Vienna and established the average incubation period at nine days.

Symptoms.—The first symptom to appear is a rise in temperature. The temperature may go as high as 42° C. The animal is depressed and stands apart from other animals with the head dropped and back arched. In some cases there is a short period of excitement which lasts for a few hours. The appetite is diminished, but thirst may be markedly increased. The urine is scanty and of a darker color. Milk secretion is diminished. Respiration and pulse are accelerated. About the second day the mucous membranes become inflamed, the conjunctivæ are red, lacrimation is increased, and the lips become swollen. There may be a brown fetid discharge from the nose, and the nasal mucous membrane is markedly inflamed and covered with minute hæmorrhages. The buccal mucous membrane is inflamed, and salivation is increased. On the surface of the membranes are noted grayish white nodules which at first are hard but later become soft. Larger patches then develop which may be wiped off. These have been called Gerlach's "caseous plaques." Eventually erosions appear, and in some cases ulcers are formed. The process also involves the mucous membrane of the intestinal tract, and at first there is constipation followed in one or two days by a profuse diarrhœa. The stools are thin, watery, fetid, sometimes bloody, and contain mucous shreds. There is a mucopurulent discharge from the vagina of cows and heifers. According to Dieckerhoff the respiratory symptoms begin with a dry, painful cough. Respiration is accelerated and there is marked vesicular breathing followed later by dry and moist râles. The heart later in the course of the disease becomes weakened. The fever reaches its height about the fifth or sixth day and then drops with the onset of the diarrhœa to normal or below normal. In some cases skin lesions appear in the form of vesicles over the back of the neck and on the scrotum. Pregnant animals frequently abort during the course of the disease. In most cases the course of the disease runs from four to seven days. Death may result the second or third day or the disease may be prolonged for fourteen to sixteen days. Convalescence lasts from two to three weeks.

Aberrant forms of cattle plague occur. For instance, Rickmann(14) in German South Africa states that cattle and other animals may be infected to imperceptible degrees. Eggerbrecht(15) states that in China cattle may only have a rise in temperature to 40° C. for two days. Littlewood(16) in Egypt observed that cattle imported from Asia Minor may not show clinical symptoms of the disease and yet at autopsy reveal lesions. Baldrey(17) believes that the virus becomes attenuated by long residence in one place. There seems to be little evidence of this fact in the Philippines where the disease has been present for over thirty years. Baldrey has described a toxin produced in broth when infected blood was added to it, but Boynton states that there is no evidence of toxin formation.

Animals susceptible to the virus of rinderpest.—Cattle, carabaos, goats, sheep, and hogs are all affected by rinderpest. Camels are said to have been artificially infected. Certain breeds of cattle are more resistant than others. The mortality varies greatly with the breeds of cattle and with the species of animal affected. In range cattle it is less than 50 per cent, while in colored breeds it may be 75 per cent. In goats the mortality is low. Usually only an elevation of temperature and in some cases a diarrhœa is noted. This was noted by Kolle and Turner(18) and by Topacio(19) and corresponds to the author's observations in the Philippines.(20)

Immunity.—Recovery from an attack of cattle plague usually results in a long-lasting immunity, in some cases for the life of the animal. Artificial immunization may be carried out by at least two methods with a high degree of success. A number of methods of immunization against rinderpest have been advanced; such as, immunizing with secretions of affected animals, with bile of affected animals, with blood serum alone, the simultaneous method (immune serum and virus), etc., but the most dependable methods are those of Boynton and of Kelser.(21) Boynton believes that the immune-serum method is too expensive and inefficient since the immunity so produced lasts for only ten to twenty-one days. Boynton's vaccine has been used in the Philippines with good success for several years and consists of ground infected tissues (lymph glands, spleen, liver, heart, kidneys, and testicles) treated with glycerin and phenol as follows: Tissue, 900 grams; glycerin, 300 cubic centimeters; and phenol, 6 cubic centimeters. The vaccine is stored in amber bottles, heated in a water bath at 42° C. for three hours, and then stored in the ice box for three to six months before using. Healthy animals receive two or three injections and are found to have developed an immunity which lasts from one to two years. This method is equally effective for Herefords, Jerseys, Guernseys, Holsteins, mixed breeds, and carabaos.

Recently Kelser and the Bureau of Agriculture of the Philippines, working in collaboration, published a method of preparing a vaccine for rinderpest which consists of ground infected tissue diluted with an equal part of physiological saline. Chloroform is added and thoroughly mixed with the vaccine just before storing in the ice box, and it is found that this vaccine may be used within a few hours after preparation while the Boynton vaccine must be "ripened" for long periods of time before use and further will keep for only a short time. In most of Kelser's experiments this vaccine was used within twenty-four hours after preparation. Vaccine prepared according to the method of Kelser has been demonstrated to retain its potency for a period of at least one year. Thus this represents a decided advantage over the Boynton method. The Kelser method is somewhat similar to a method which has been employed in Japan in recent years by Kakisaki and his associates.(22) For the destruction of the rinderpest virus in their vaccine, these investigators employ toluol and glycerin, and, further, subject the vaccine to the action of incubator temperature, 37.5° C., for seven to ten days. Toluol results in a very viscid vaccine which offers difficulty in administration, and, further, it is absorbed very slowly.

Rodier(23) has prepared a vaccine consisting of ground lymph gland, tonsils, and spleen. The tissue is then treated as in the method described by Kelser. In Rodier's opinion a vaccine prepared from these tissues is much more potent than vaccine prepared from other tissues and preliminary experiments have demonstrated that one injection of 15 cubic centimeters (7.5 grams) will protect carabaos against rinderpest.

To summarize the present status of the immunization of susceptible animals against rinderpest with tissue vaccine Kelser(24) states:

> A chloroform-treated vaccine, consisting of a suspension of finely ground tissues (lymph glands, tonsils, spleen, liver) from animals killed in the acute stages of rinderpest is highly efficacious. Such vaccine can be used shortly after preparation, offers no technical difficulty in administration, and possesses excellent keeping qualities.
>
> Cattle and carabaos are solidly immunized against the disease when given three, weekly injections of such vaccine in amounts of 15 to 20 cubic centimeters. For the immunization of cattle, experimental evidence indicates that in practice the number of injections of this vaccine can actually be reduced to one with satisfactory results. However, for the immunization of carabaos, if a single dose of vaccine is to be employed

it is desirable, in the preparation of vaccine for such animals, to use only the tissues of very highest potency (lymph glands, tonsils, spleen), leaving out the less potent liver tissue. This is necessary because of the extreme susceptibility of carabaos to rinderpest. Where such procedure is followed it is entirely possible to satisfactorily immunize carabaos with a single injection of vaccine. A number of experimental and field tests along this line by Rodier have fully established this point.

Pathology.—At autopsy animals dead of cattle plague show the most important changes in the mucous membranes, especially those of the gastrointestinal tract. The mucous membranes are dark red and covered with small hæmorrhages and in some parts with a pseudodiphtheritic membrane. In the intestines the follicles are swollen and contain caseous material. Peyer's patches are quite similar. The inflammation is especially evident in the fourth stomach, in the region of the ileocæcal valve, and the rectum. A marked stomatitis may be present together with erosions and in some cases ulceration of the mucous membranes of the mouth. The large intestines may show only an acute catarrh. The liver shows parenchymatous or fatty degeneration, and the gall bladder is distended. The spleen may be swollen and show little change other than this. The kidneys show cloudy swelling and fatty degeneration. The lungs are hyperæmic and may show evidences of a catarrhal pneumonia. The bronchi may be filled with a pale yellow exudate. The heart is flabby and shows hæmorrhages under the endocardium and epicardium. The lymph glands are swollen and the brain shows moderate hyperæmia and œdema in the white substance. In severe cases hæmorrhages have been noted in the marrow of the long bones.

Control measures in rinderpest.—Quarantine against countries where rinderpest is prevalent, and isolation of affected animals are indicated in controlling this disease. Prophylactic vaccination is indicated in countries where the disease is present. The surest method of eradication is to slaughter all affected animals where this procedure is possible.

BIBLIOGRAPHY

1. Nicolle and Adil-Bey, A. P. 13 (1899) 329; 16 (1902) 56.
2. Nocard, Quoted from Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals. 3d Eng. ed., Mohler and Eichhorn. Alexander Eger, Chicago (1926).
3. Koch, Zbl. f. Bakt. 21 (1897) 526.
4. Kolle, Z f. Hyg. 27 (1898) 45.
5. Theiler, D. t. W. (1898) 205.

6. BALDREY, Journ. Trop. Vet. Sci. **6** (1911) 169, 251; Agr. Journ. India **7** (1912) Pt. 3, 294.
7. BRADDON, Parasitology **6** (1913) 265–275.
8. BOYNTON, Philip. Journ. Sci. **8** (1913) 509–521; Philip. Bur. Agr. Bull. 29; Philip. Journ. Sci. § B **9** (1914) 39–44; 259–268; Philip. Agr. Rev. **10** (1917) 272, 410; Philip. Journ. Sci. § B **13** (1918) 95–121, 152; Philip. Agr. Rev. **15** (1922) 218–228; Philip. Journ. Sci. **36** (1928) 1.
9. RÉFIK-BEY, Ann. d. l'Inst. Pasteur **13** (1899) 596; **16** (1902) 163.
10. EDINGTON, Lancet **1** (1899) 357.
11. STOCKMAN, Ann. Rpt. Transvaal Dept. Agr. (1903–04) 67; Journ. Comp. Path. **18** (1905) 207.
12. YERSIN, Bull. Econom. n. s. **6** (1904) 241.
13. RUEDIGER, Philip. Journ. Sci. § B **3** (1908) 165; 147; § B **4** (1909) 381.
14. RICKMANN, Tierzucht und Tierkrankheiten in Deutsch Suedwest Africa (1908) 156.
15. EGGERBRECHT, Z. f. Infkr. Haustiere **7** (1910) 54.
16. LITTLEWOOD, Journ. Comp. Path. and Therap. **18** (1905) 312.
17. BALDREY, Journ. Trop. Vet. Sci. **6** (1911) 169, 251.
18. KOLLE and TURNER, Z. f. Hyg. **27** (1898) 45.
19. TOPACIO, Philip. Agr. Rev. **15** (1922) 229–236; **19** (1926) No. 4.
20. MCKINLEY, Proc. Soc. Exp. Biol. & Med. **26** (1928) 22.
21. KELSER, Journ. Am. Vet. Med. Assoc. n. s. **24** (1927) 97; The Military Surgeon (July, 1927); paper at meeting of F. E. A. T. M. Calcutta (December, 1927).
22. KAKIZAKI, NAKANISHI, and NAKAMURA, Journ. Jap. Soc. Vet. Med. **6** (1927) 107–120.
23. RODIER, personal communication (1927).
24. KELSER, personal communication (1928).

OTHER REFERENCES

KELSER, Manual Vet. Bact. Williams and Wilkins, Baltimore (1927).
HUTYRA and MAREK, Spezielle Pathologie und Therapie des Haustiere. G. Fischer, Jena (1910).
WARD, WOOD, and BOYNTON, Philip. Journ. Sci. § B **9** (1914) 49–79.
HUTYRA and MAREK, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Eng. ed., by Mohler and Eichhorn. Alexander Eger, Chicago (1926).

VESICULAR STOMATITIS OF HORSES

Definition.—Vesicular stomatitis is an acute, febrile, infectious disease of solipeds that is characterized by a vesicular eruption on the tongue, mucous membranes of the cheek and lips, and sometimes on the skin surrounding orifices.

History.—Vesicular stomatitis was first observed in 1884 in South Africa as an affection of horses. Early descriptions of the disease were reported by Hutchen and by Theiler. The disease did not appear in the United States until 1916 when cases where discovered among the army horses and mules about to be shipped to Europe. It is thought that the shipment of horses and mules to Europe during the World War was responsible for the introduction of the disease into France, England, and Italy.

The virus of vesicular stomatitis.—The virus of vesicular stomatitis is present in the vesicles and in the saliva of affected animals. With this material the disease may be easily transmitted to susceptible animals. It is thought that the infection usually is transmitted by feeding from common troughs and enters the susceptible host through cuts and abrasions of the lips and buccal mucous membranes. In France cattle also became infected, and in some cases the lesions of the disease resembled remarkably the lesions of foot-and-mouth disease. In the United States the disease also spread to cattle though it did not infect swine. The marked similarity of the lesions of vesicular stomatitis and foot-and-mouth disease suggested that both diseases might be of a common origin and in fact might be one and the same disease. However, the disease as it appeared in cattle did not involve the hoofs or the udders. Mohler(1) in 1918 and Cotton(2) in 1926 reported that the virus of vesicular stomatitis is not fiterable through filters that retain *B. prodigiosus.* It is, of course, well established that the virus of foot-and-mouth disease is filterable, and the difference in the filterability of these two viruses appeared to be a point which could be well used for differentiating these two diseases one from the other. However, Olitsky, Traum, and Schoening(3) have only recently shown that the virus of vesicular stomatitis is filterable through Berkefeld V and N candles, through Seitz asbestos disks, and through Chamberland bougies L3 and L7. Cotton(4) in a report published about the same time reports that the virus of vesicular stomatitis, according to his experiments, is filterable through V, N, and W grades of Berkefeld, bacteria-retaining filters. These two reports based upon experimental infection in guinea pigs, appear to establish the filterability of this virus beyond doubt. Further, Olitsky, Traum, and Schoening have shown that in guinea pigs cross-immunity tests with the virus of vesicular stomatitis and the virus of foot-and-mouth disease are negative. The same results were obtained in cattle. Swine are also said to be susceptible to the virus, and no cross immunity can be demonstrated in this animal between vesicular stomatitis and foot-and-mouth disease. These authors also showed that while the horse is highly sensitive to the virus of vesicular stomatitis when inoculations are made upon the tongue, this animal is resistant to foot-and-mouth disease when inoculated on the tongue, mucous membranes of the lips, or intramuscularly. They regard the horse as the best test animal for differentiating between the two diseases.

Incubation period of vesicular stomatitis.—In experimental vesicular stomatitis in horses the incubation period is very short, ranging from thirty-six to seventy-two hours. Swine show a marked rise in temperature twenty-four to forty-eight hours after the injection of the virus. In cattle the incubation period in experimental infections is about forty-eight hours. Typical lesions are produced in the scarified hind pads of guinea pigs within thirty to forty hours following inoculation.

Symptoms of vesicular stomatitis in horses.—The first symptom of the disease to appear is a rise in temperature. This is followed by the early appearance of pea-sized vesicles on the tongue and in some cases upon the mucous membranes of the mouth and on the lips. The vesicles quickly rupture and leave a denuded area. Practically the entire surface of the tongue may be denuded of its epithelium. The tongue is blue (blauw tong) and may be covered with frothy saliva. For a short time the animal is unable to eat, but the lesions rapidly heal and recovery follows within three or four days. The outcome of the disease is practically always favorable. The author has been unable to find any cases of fatality on record.

Animals susceptible to the virus of vesicular stomatitis.—Horses are chiefly affected by this disease, but cattle may also be infected naturally. Experimentally swine and guinea pigs may also be infected. According to Vallée and Carré(5) transmission to sheep does not take place.

Immunity in vesicular stomatitis.—One attack of the disease produces an immunity to subsequent infections. Since the disease is so mild it has been recommended that animals be artificially inoculated in order to prevent its spread and to save working time.

BIBLIOGRAPHY

1. MOHLER, Journ. A. V. M. A. 51 n. s. 5 (1918) 410–422.
2. COTTON, Journ. A. V. M. A. 69 n. s. 22 (1926) 313–332.
3. OLITSKY, TRAUM, and SCHOENING, Journ. A. V. M. A. 70 n. s. 23 (1927) 147–167.
4. COTTON, Journ. A. V. M. A. (1927) 168–184.
5. VALLÉE and CARRÉ, C. R. de l'Acad. de Sc. 174 (1922) 207.

INFECTIOUS PUSTULAR STOMATITIS OF HORSES

Definition.—Infectious pustular stomatitis is a benign acute contagious disease of horses. It is characterized by a pustular eruption upon the mucous membrane of the mouth which in some cases develops into ulcers.

History.—In 1878 infectious pustular stomatitis was first described under this name by Eggeling and Ellenberger.(1) Prior to that date the disease had been confused with foot-and-mouth disease, with coital exanthema, and with horsepox. Based upon the work of the above authors this disease to-day is recognized as a disease entity, although workers in England and France are still prone to consider the disease a form of horsepox. The experimental work of Eggeling and Ellenberger, however, has been confirmed by Freidberger.(2)

The virus of infectious pustular stomatitis.—The virus of infectious stomatitis is present in the early nodules and resulting pustular lesions and also in the secretions of the mucous membrane and in the saliva of infected animals. It is filterable. The disease can be transmitted by rubbing infected saliva upon the scarified skin or mucous membrane of suceptible animals.

The fact that by some investigators this disease is still thought to be a form of horsepox has been mentioned above. There is some experimental evidence to support this concept. For example cowpox virus when inoculated on to the skin of a horse, according to De Jong, will produce lesions similar to those of infectious pustular stomatitis, though a horse which has previously had infectious pustular stomatitis will not react to the vaccine virus, thus indicating an immunity. The stomatitis virus also produces lesions similar to cowpox in calves, rabbits, and cattle. Guarnieri bodies were demonstrated by the above author upon the cornea of rabbits infected with the virus of stomatitis. Further De Jong vaccinated nine children with material derived from a horse affected with stomatitis, and in eight of the children typical vaccinia developed in from five to seven days while in the ninth child, who had been previously vaccinated, the virus did not take.

From these experiments we may conclude that there is a possible relationship between the viruses of stomatitis and cowpox, but for the time being, since opinion of investigators is divided regarding the matter, we are not justified in considering stomatitis and cowpox or horsepox one and the same disease.

In natural infection the disease does not spread to other species of animals, such as domestic cattle.

Incubation period in infectious pustular stomatitis.—In natural infections the period of incubation in infectious pustular stomatitis is about eight days. In artificial infections it is somewhat shorter, ranging from three to five days.

Symptoms.—The disease begins with a moderate rise in temperature of one or two degrees. Evidently the mucous membrane of the mouth is sensitive, for the animals feed much more slowly and are apparently relieved when the snout is in water. Salivation begins early, and saliva may be seen dropping from the corners of the mouth. On the mucous membranes of the mouth there first appear small red spots which later develop into nodules, in turn changing into vesicles and pustules, the entire process requiring about six days. The pustules may later turn into ulcers which extend down to the submucosa. The lesions of the disease may also involve the nasal mucous membrane and the eyes (Tannenberger(3)) or may occur upon the skin (Dieckerhoff(4)). If the skin is involved, the hair may fall out. If the eruption is generalized over the body, greater or lesser degrees of emaciation may result. The ulcerations heal gradually with the formation of fine scar tissue.

The disease usually runs its course within ten to fourteen days but may extend for three or four weeks. As a rule animals recover from the disease, though occasionally death follows as a result of secondary infection. Vesicular stomatitis may somewhat resemble pustular stomatitis in its vesicular stage, but in the former disease no nodules are formed preceding the appearance of vesicles.

Animals susceptible to the virus of infectious pustular stomatitis.—The disease is primarily a disease of horses, but according to Eggeling and Ellenberger the disease may also be transmitted to cattle, sheep, hogs, and chickens. Schultz(5) states that man may also become infected.

Immunity.—Younger animals are more susceptible to the disease than older animals. Since the disease is self-limited and epidemics do not spread for any considerable distances, it is believed that the virus becomes attenuated during its passage from one animal to another. One attack of the disease confers immunity to subsequent attacks of the disease for indefinite periods of time. Inoculation of healthy animals with the stomatitis virus is practiced successfully in preventing the spread of the disease and shortening its duration.

Control measures.—Isolation and quarantine of infected animals and exposed cases together with artificial inoculation of the virus into healthy animals are the measures indicated for the control of this disease.

BIBLIOGRAPHY

1. EGGELING and ELLENBERGER, A. f. Tk. 4 (1878) 334.
2. FRIEDBERGER, D. Z. f. Tm. 5 (1879) 265.
3. TANNERBERGER, Ost. W. (1912) 65.
4. DIECKERHOFF, Spez. Path. (1892) 1 (1892) 391.
5. SCHULTZ, Münch. med. Woch. (1894) 201.

OTHER REFERENCES

HERTWIG, Mg. 8 (1841) 305.
BOULEY, Dict. de méd. vét. 9 (1871) 451.
GRÖNER, Vet. Jhb. (1895) 97.
POSCHL, A. L. (1904) 681.
REHNITZ, S. B. (1906) 176.
MORI, Clin. Vet. (1909) No. 42.
OSTERTAG and BUGGE, Zt. Infectionskrank Haust. 1 (1905–06) 3.
HUTYRA and MAREK, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Eng. ed., by Mohler and Eichhorn. Alexander Eger, Chicago (1926).

EPHEMERAL FEVER OF HORSES

THREE-DAY SICKNESS; STIFF SICKNESS

Definition.—Ephemeral fever is an acute, febrile infection of horses which closely resembles African horse sickness but is distinguished from that disease by its short incubation period and short duration of four or five days. It is characterized by a rapid rise and fall in temperature that lasts only a few days associated with weakness and slight congestion of the conjunctiva.

History.—Ephemeral fever was described in South Africa by Schweinfurth in 1867 in cattle, in 1906 by Edmonds, and in 1910 by Theiler(1) in horses.

Distribution.—This disease occurs in South Africa in areas in which African horse sickness occurs and has been described in Rhodesia, Natal, and in the Cape colonies.

Incubation period.—The incubation period in ephemeral fever is two to three days.

Symptoms.—After the two to three days' incubation period there is a sudden rise in temperature, conjunctivitis, frequent respiration, loss of appetite, weakness, weak pulse, distinct venous pulse, and constipation. After four or five days the temperature falls suddenly. The outcome of the disease is usually favorable, but death results in about 3 per cent of cases, usually caused by a terminal pneumonia.

The virus of ephemeral fever.—The virus is unknown. It may be transmitted to healthy animals by injecting the blood of infected cattle. It may also be transmitted to healthy animals by injecting them either subcutaneously or intravenously with preserved or filtered blood or serum. It falls within the group of filterable viruses.

Immunity.—A single subcutaneous injection of virulent blood will produce a lasting immunity against subsequent attacks of the disease but will not protect against African horse sickness. In some cases, however, there has been evidence that the immunity so produced is transient, since the animals again reacted to the virus when injected a second time with the virus at a later date.

This disease has been confused with three-day fever, or stiff sickness, and the relationship is not exactly known. In three-day fever a temporary immunity only is produced by virtue of having the disease, and is said to last only about six weeks. Three-day fever in cattle resembles three-day fever, pappataci fever, in man, and is also quite similar to dengue fever in man. The possibility of mosquito transmission has been considered, but there is no evidence that the disease is transmitted by this vector. While Theiler described ephemeral fever as occurring in horses, the three-day fever, or stiff sickness, also is found in cattle. For the present it is best to consider these diseases together, although future investigations may bring out new facts by which they may be differentiated.

Reference 1. THEILER, V. J. (1910) 587.

EQUINE INFLUENZA: INFLUENZA CATARRHALIS

INFLUENZA ERYSIPELATOSA, LEUMA, PINK EYE, TYPHOID FEVER; PFERDESTAUPE (GERMAN); GRIPPE, FIEVRE TYPHOIDE, PASTEURELLOSE DU CHEVAL (FRENCH)

Definition.—Equine influenza is an acute, febrile, contagious disease of horses which is characterized by catarrhal inflammation of the mucous membranes, inflammatory swellings of the subcutis, and inflammation of the tendons. At times this disease is spoken of as "shipping fever," but contagious pneumonia and strangles are also included by this term.

History.—Equine influenza has been studied since early in the last century. Until quite recently its nature was not clearly understood. It was known to be frequently complicated by pleuropneumonia, and the disease was not recognized as an entity. As a result the history of the disease is obscure in as much as early reports on the disease are very confusing, and the particular disease described is not characteristic of uncomplicated influenza in horses as it is known to-day.

Distribution.—The disease may be said to be general in its distribution, since it has been reported far and wide in various parts of the world. It may occur sporadically or in epidemic form and in the latter case spreads rapidly through its susceptible host over wide areas.

Incubation period.—The average incubation period in equine influenza is from four to six days. In experimental infections it varies from forty hours to ten days and may even extend to two weeks. When the disease follows coitus it develops within six to nine days.

Symptoms.—The disease begins with a rise in temperature associated with rapid pulse and respiration. The animal becomes depressed, is easily fatigued, and loses its appetite; it moves with a sluggish gait and frequently staggers. It prefers to stand still with the eyes half closed and the ears drooping. The temperature mounts to 41° C. or higher. The conjunctiva becomes reddened, and the eyelids swell. There is photophobia and increased lacrimation, and iritis may develop.

As the catarrhal condition of the respiratory passages develops there is a dry, painful cough. Nasal catarrh may become marked and the salivary glands enlarged. The tongue is dry and coated. A gastric catarrh frequently develops, and the early constipation is usually followed by diarrhœa. The urine is scanty and dark yellow, often containing albumin and bile pigments. In females the catarrhal condition may also involve the vagina from which there is a mucopurulent discharge. In some epidemics œdema of the subcutis is a prominent symptom and in such cases there is usually an inflammation of the tendons. In severe forms of the disease the nervous system may become involved. Such cases are characterized by deafness, paralysis, and chorealike movements, and after death hæmorrhages are found in the meninges of the spinal cord.

The disease may be complicated by pneumonia, and then it usually terminates in death. In such a case the heart and kidneys are markedly impaired. Uncomplicated equine influenza usually runs its course within a week and the majority of the affected animals recover.

Animals susceptible to the virus of equine influenza.—The disease is limited to solipeds. It is not influenced by the age or sex of the animal.

The virus of equine influenza.—In 1882 Dieckerhoff(1) transmitted the disease to healthy animals by subcutaneous and intravenous injection of fresh blood from infected animals. About the same time Poels succeeded in infecting healthy animals with fresh and filtered semen from a stallion that had infected mares during the act of coitus. He also showed that filtered blood from infected horses was capable of inducing the disease in healthy animals. In 1911 Basset(2) infected healthy animals with filtered blood and with blood serum that had been kept in the ice box for four months. Gaffky(3) during the next year established the incubation period for the disease in artificially infected animals. Following subcutaneous injection of 5 cubic centimeters of infected defibrinated blood the disease developed within five to six days. Intravenous injection induced the disease within four days. Inoculation with filtered blood serum also gave positive results. Basset found the blood of a horse that had recovered from the disease virulent after three months.

In the natural infection the direct transmission of the disease from horse to horse seems to be the most frequent method of spread of the disease. Stable-fly transmission and inhalation infection have been advanced as possible methods of spread, but there is little evidence to substantiate these theories. Since the virus is present in the blood stream of infected animals and in secretions such as semen, it seems more likely that the disease may spread by contamination of drinking water and food as well as by direct contact. It appears quite likely that healthy animals may act as carriers for the virus, especially animals that have recovered from the disease.

Immunity in equine influenza.—One attack of the disease confers a long-lasting immunity. Basset was unable to reinfect recovered animals with virulent blood. Rarely, however, second attacks do occur. This is interesting to note, since in former times this disease was associated with pleuropneumonia and both were thought to be one and the same disease. An animal recovered from influenza, while immune to second attacks of this disease, is still susceptible to pleuropneumonia and vice versa. The two diseases are not related except as one may complicate the other.

No vaccine or antiserum prepared for the prevention of this disease has offered much encouragement. While numerous products have been placed on the market and used extensively, none of them has in any way proved of value for the prevention and control of equine influenza.

Pathology.—The anatomical changes as a result of equine influenza consist chiefly in reddening, swelling, and sometimes infiltration of the mucous membranes, parenchymatous degeneration and swelling of the kidneys, liver, and heart muscle, and moderate swelling of the spleen and lymph glands. The intestinal mucosa shows a catarrh, and there may be ulcers and erosions. The lungs show an acute œdema, hyperæmia, and in complicated cases a marked pneumonia. The tendons may be inflamed and the surrounding tissue markedly infiltrated with a clear serous fluid.

Control measures in equine influenza.—Isolation may be practiced as a preventive measure, but usually it seems more practical to infect artificially the entire stable and let the disease run its course. This method has the danger of transmitting infectious anæmia from animal to animal with the blood.

BIBLIOGRAPHY

1. DIECKERHOFF, D. Pferdestaupe, Berlin (1882).
2. BASSET, C. R. **153** (1911) 485; Rec. (1912) 88.
3. GAFFKY, Z. f. Vk. **24** (1912) 212.

OTHER REFERENCES

KELSER, Manual of Veterinary Bacteriology. Williams & Wilkins, Baltimore (1927).
SPINOLA, D. Influenza d. Pferde, Berlin (1844).
FALKE, D. Influenza s. Pferdes usw., Jena (1862).
SIEDAMGROTSKY, Sacks. Jhb. (1891–93).
SCHUTT, B. t. W. (1912) 59.

INFECTIOUS ANÆMIA OF HORSES

SWAMP FEVER OF HORSES; MALARIAL FEVER OF HORSES; RIVER-BOTTOM DISEASE

Definition.—Infectious anæmia of horses is an acute infectious disease of horses caused by a filterable virus. It is characterized by a septicæmia, a gradual destruction of red blood cells, weakness, and fever.

History.—Infectious anæmia of horses has been recognized as a disease entity since the middle of the last century. The infectious nature of this disease was indicated by Zschokke(1) in 1883, although the cases described by this author were spoken of as pernicious anæmia at that time. Later a similar disease, now thought to have been the same disease, was described in 1886 by Fröhner(2) and in 1890 in Germany by Ostertag.(3) However, it was not until 1906 that Carré and Vallée(4) demonstrated the true nature of the disease by experimental work which was later amply confirmed by various investigators. (Hempel;(5) Ostertag; Van Es, Harris, and Schalk;(6) Fröhner; and Abderhalden and his associates.(7))

Distribution.—Infectious anæmia of horses has been described in France, Germany, Hungary, Sweden, Switzerland, Austria, Russia, Japan, North America, South Africa, Australia, and the Indies. Its distribution may be said to be quite general in all parts of the world.

The virus of infectious anæmia of horses.—According to the investigations of Carré and Vallée the infectious agent of this disease is filterable through the pores of both Chamberland and Berkefeld filters. Up to the present time all cultural methods which have been tried have failed. The virus is present in the blood stream of infected animals, in serum, and in washed red blood cells, in organs such as the spleen, liver, kidneys, spinal cord, lymph glands, lungs, salivary glands, and in such tissues as muscle and bone marrow. The virus is also found in the milk, intestinal contents, urine, and tears. According to Ostertag the saliva is not infectious. Japanese scientists have described a spirochæte, *Spirochæta equi-infectiosa,* as the etiological agent in this disease, but other investigators have been unable to confirm this work. Intracellular bodies have been described as occurring in liver cells and within mononuclear cells which resemble parasites, but their nature has not been determined.

The virus is very sensitive to heat, being destroyed in from forty to fifty minutes at 60° C. Low temperatures have little effect upon it. It is destroyed by sunlight. The virus remains viable for about three months in blood and urine. By subcutaneous injections of 0.01 cubic centimeter of virulent blood into susceptible animals the disease is produced, though large amounts of virus by mouth are necessary to produce symptoms.

Incubation period in infectious anæmia of horses.—In artificial infection the incubation period ranges from five to thirty days. In some cases the period of incubation is only one or two days, while in exceptional cases it may last for weeks. The usual average given is seventeen days. In the natural infection the incubation period ranges from eighteen to twenty-five days. The longest period of incubation noted for this disease was fifty-seven days.

Symptoms.—At the beginning of the disease there is marked weakness and depression of the animal. The weakness is especially noted in the hind legs. The animal may stagger, trip and fall down, and rise with great difficulty. There is a gradual rise in temperature which may go as high as 42° C. Usually it is somewhat lower than this, and the fever may be of an intermittent character. Pulse and respiration are also increased following the temperature curve. The mucous membranes of the eyes, nose, mouth, and vagina appear pale and yellow, and may present minute hæmorrhagic areas. In some cases a diarrhœa develops that may be tinged with blood. Urination is frequent, and the urine is found to contain albumin. The spleen is enlarged and may be palpated rectally. The appetite is usually diminished, in some cases it may be completely lost, and there results a rapid emaciation of the animal. In the course of two weeks the blood contains a markedly diminished number of red blood cells. This may reach less than one-half the normal number of red corpuscles. In proportion to the duration of the disease lymphocytosis develops which may reach 40 to 70 per cent. Howell-Jolly bodies are found in the red blood cells. While poikilocytosis is not seen, anisocystosis is frequently observed and occasional basophilic granulation may also be present. In most cases the color index is not increased to any great extent. The blood picture in general contains nothing typical of the disease that will serve to differentiate it from other forms of anæmia. As the disease progresses the weakness becomes more apparent, and paralysis of the hind legs gradually comes on. Pregnant animals usually abort. The acute form of the disease lasts from five to fifteen days in younger animals and in older animals may last for three or four weeks.

Subacute, chronic, and latent forms of the disease have been described, but all are subject to acute relapse and the animal may die suddenly as a result. The acute cases usually die, while the other forms of the disease may be prolonged for long periods with intervals of apparent returning good health, but in most cases this is followed by relapse. While complete cures are very rare, the mortality is usually given as raging from 30 to 70 per cent.

Immunity.—Little, if any, natural immunity exists for this disease among the Equidæ. Horses, mules, and donkeys are all susceptible to the virus of infectious anæmia. There is some evidence that some breeds apparently possess more resistance than others, but beyond this there is no indication of natural immunity. There is no reason to suppose that one attack of the disease will protect animals against future attacks. Any immunity gained in this manner is of a short duration as indicated by the relapses so frequently occurring in chronic forms of the disease that endure for months. No suitable method of producing artificial immunity has been placed on a satisfactory basis. Virus suspended in serums from nonsusceptible animals is apparently markedly weakened so that no immunity is produced when such preparations are injected into a susceptible animal; or an animal becomes infected following such injections and remains a potential agent for the spread of the disease to other animals.

Control of infectious anæmia of horses.—Isolation of the infected animal, slaughter, and proper disposal of the carcass is indicated. Quarantine of healthy animals introduced into new areas is advisable. While there seems to be sufficient evidence that the disease is spread directly from one animal to another, the control of flies and other blood-sucking insects as possible vectors has been emphasized as a contributing control measure. Due to the markedly infectious nature of this disease great care should be exercised in eliminating animals infected with this virus for use as serum producers for other diseases.

BIBLIOGRAPHY

1. ZSCHOKKE, Schw. A. 25 (1883) 11.
2. FRÖHNER, A. f. Tk. 12 (1886) 382.
3. OSTERTAG, Monh. 1 (1890) 127.
4. CARRÉ and VALLÉE, Rev. Gén. 8 (1906) 593.
   VALLÉE, Bull. (1907) 526.
5. HEMPEL, Zeit. f. Infkr. 5 (1909) 381.
6. VAN ES, HARRIS, and SCHALK, North Dakota Agr. Exp. Station, B. 94 (1911) (lit.).
7. ABDERHALDEN and BUCHAL, A. f. Tk. 37 (1911) 300.
   ABDERHALDEN and FREI, A. f. Tk. 36 (1910) 425.

OTHER REFERENCES

HUTYRA and MAREK, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Eng. ed., by Mohler and Eichhorn. Alexander Eger, Chicago 3 (1926) (lit.).

ILLUSTRATIONS

PLATE 15

FIG. 1. Foot-and-mouth disease. Salivation. (After Mohler; from Hutyra and Marek.)

2. Foot-and-mouth disease. Vesicles with erosions covered by scabs on the teats of the udder. (From Hutyra and Marek; after Moulage.)

PLATE 16

FIG. 1. Foot-and-mouth disease. Separation of the coronary band from the horny part. Vesicle on the posterior end of the interdigital space. (After Hutyra and Marek.)

2. Foot-and-mouth disease. Vesicles and ulcerations on the gums, the latter also on the nostrils. (After Hutyra and Marek.)

PLATE 17

Foot-and-mouth disease. Subepithelial vesicles on the point, the body, and the thick portion of the tongue. (After Hutyra and Marek.)

PLATE 18

FIG. 1. Hog cholera. Pig affected for two weeks. (After Hutyra and Marek.)

2. Hog cholera. Follicular ulcers with elevated borders of concentric necrotic layers. Concentric layers, protruding necrosis, and small hæmorrhages. (From Hutyra and Marek.)

3. Hog cholera. Intestine of hog with follicular ulceration. (After Hutyra and Marek.)

PLATE 19

FIG. 1. Rinderpest. Diffused necrosis with softening and localized desquamation of the epithelium on the gums; erosions (English report). (From Hutyra and Marek.)

2. Rinderpest. Reddening and partial desquamation of the epithelium on the gums of the incisors; back of the teeth a roundish and below an elongated deposit of fibrin. Some corn-shaped papillæ denuded of epithelium and therefore reddened on the point or on the sides; above to the left the epithelium of a papilla showing a belt-shaped necrosis.

PLATE 20

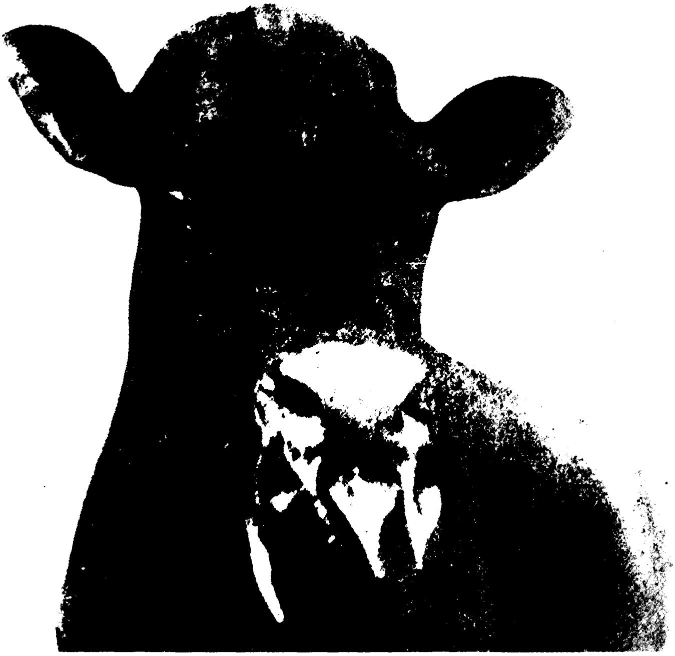
Rinderpest. Hyperæmia and swelling of the mucous membrane of the abomasum with small ulcers and scabs. (After Hutyra and Marek.)

PLATE 21

Rinderpest. Gall bladder. Acute inflammation of the mucous membrane with swelling and subsequent necrosis of the follicles. (After Hutyra and Marek.)

PLATE 22

Infectious stomatitis. Nodules, pustules, and ulceration. (After Hutyra and Marek.)



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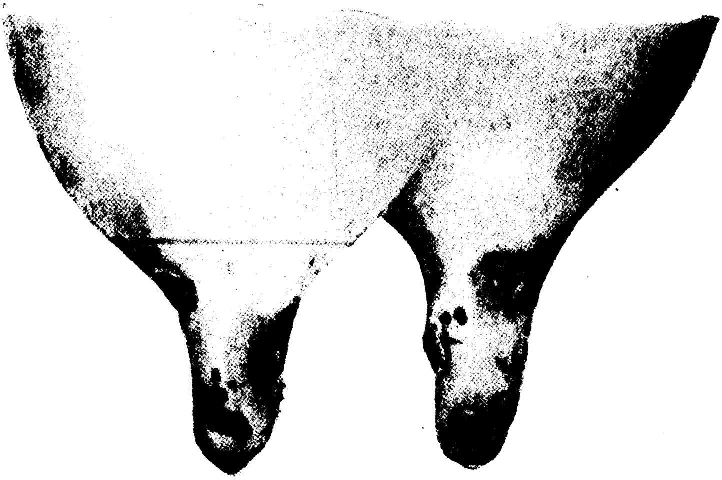

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Fig. 1. Salivation. 2. Vesicles with erosions covered by scabs on the teats.

PLATE 15. FOOT-AND-MOUTH DISEASE.

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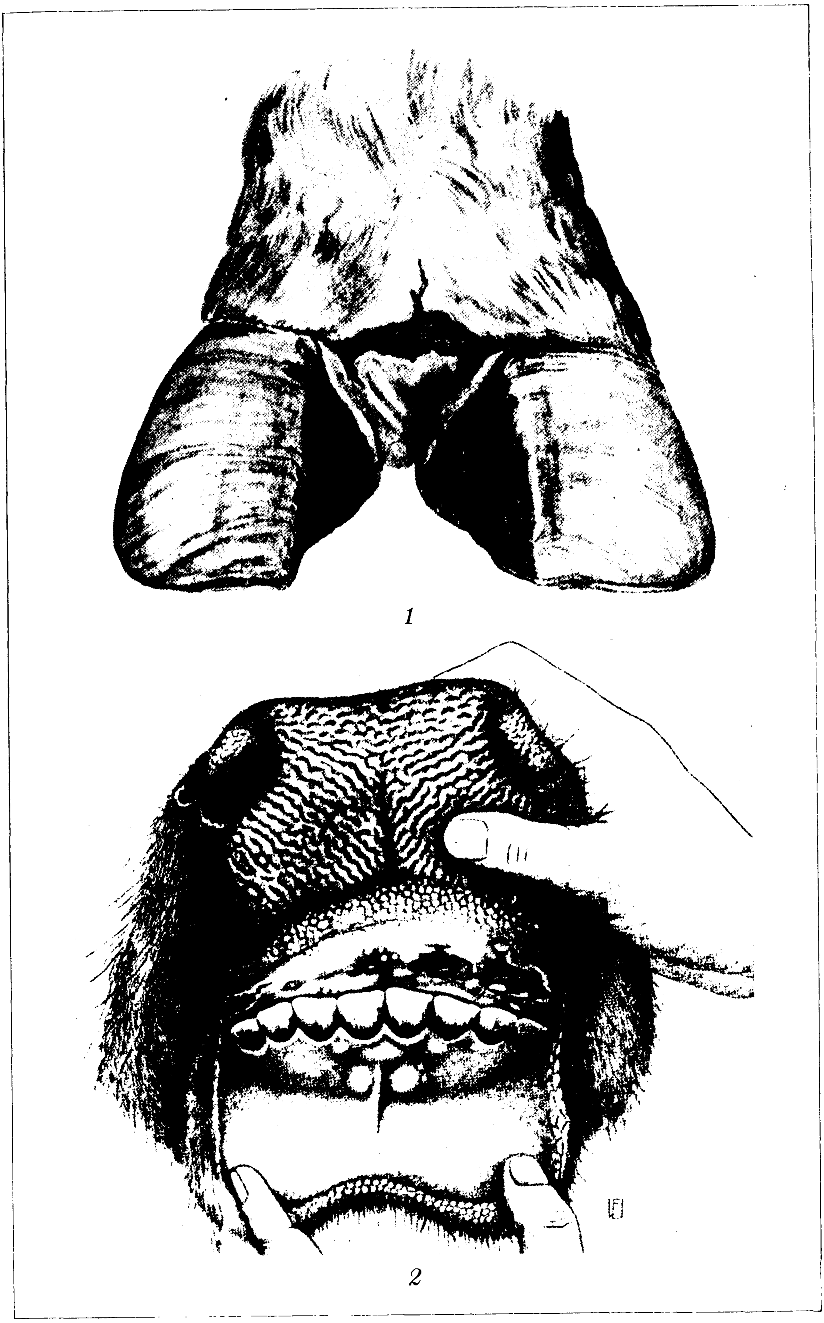

PLATE 16. FOOT-AND-MOUTH DISEASE.

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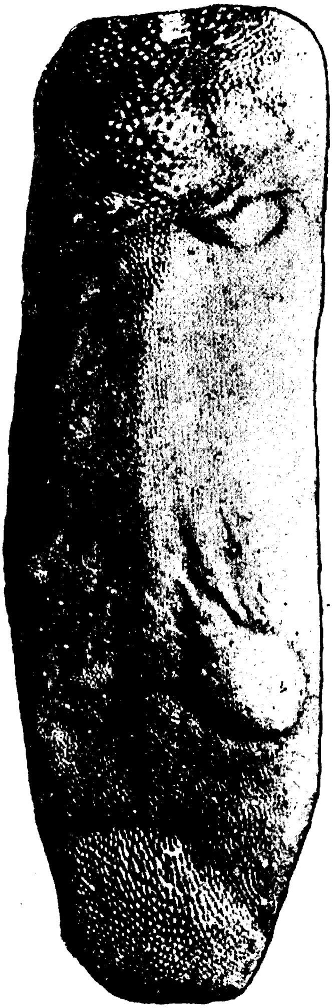

PLATE 17. SUBEPITHELIAL VESICLES ON THE POINT, THE BODY, AND THE THICK PORTION OF THE TONGUE IN FOOT-AND-MOUTH DISEASE.

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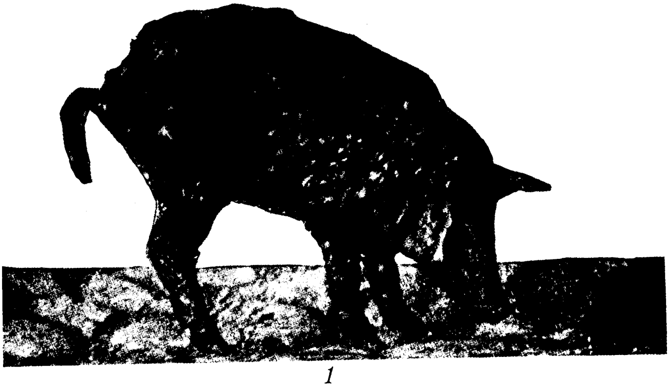

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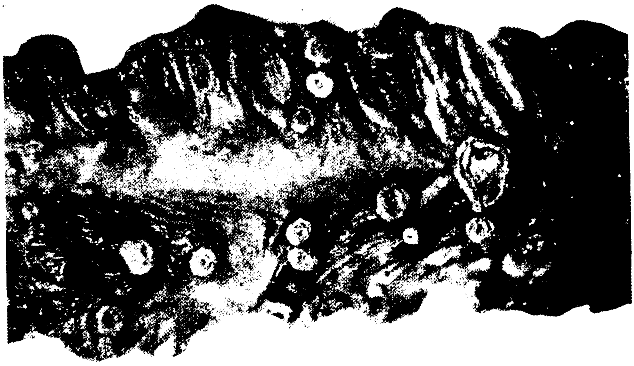

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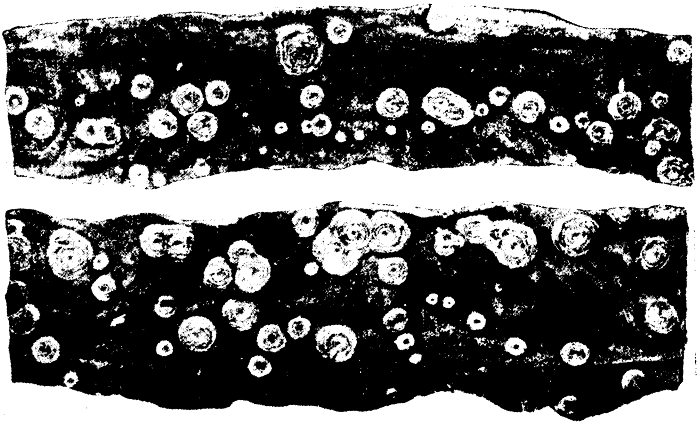

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Fig. 1. Pig affected for two weeks. 2 and 3. Follicular ulceration.

PLATE 18. HOG CHOLERA.

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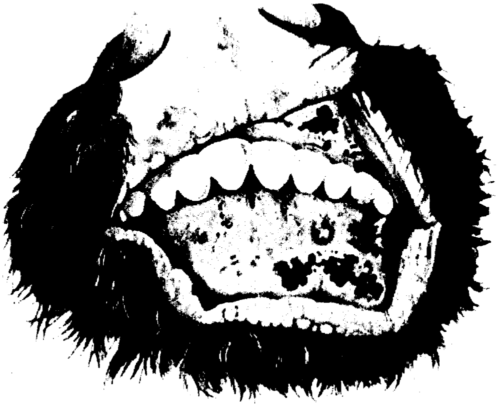

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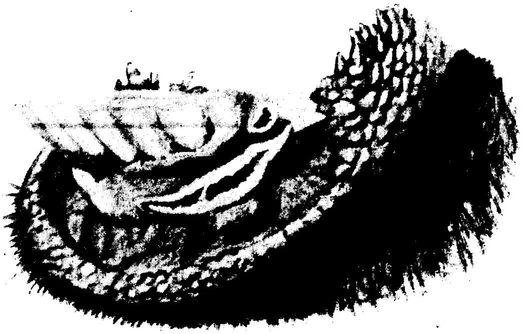

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PLATE 19. RINDERPEST.

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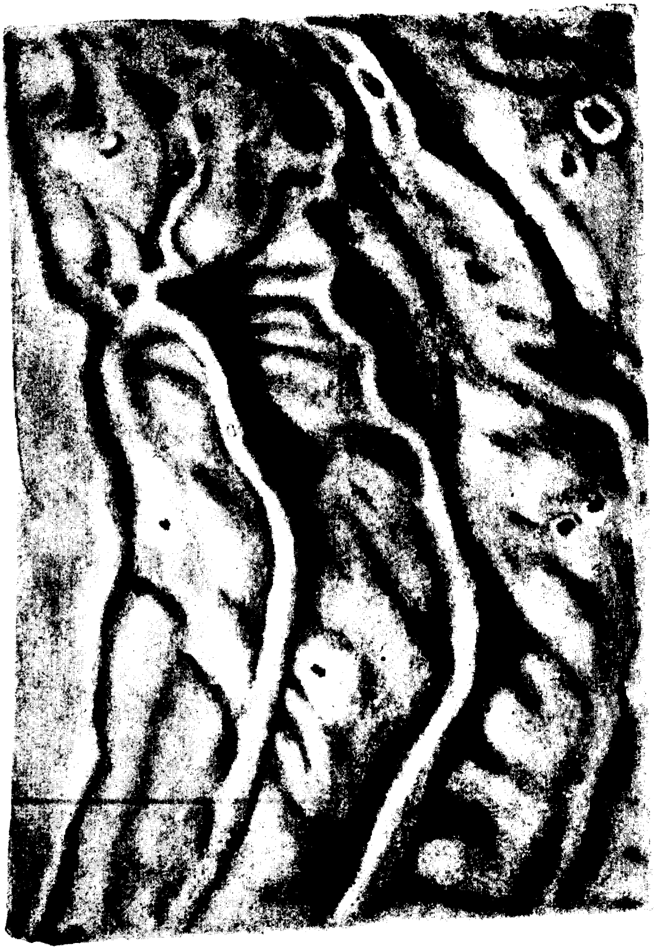

PLATE 20. RINDERPEST. HYPERÆMIA AND SWELLING OF THE MUCOUS MEMBRANE OF THE ABOMASUM WITH SMALL ULCERS AND SCABS.

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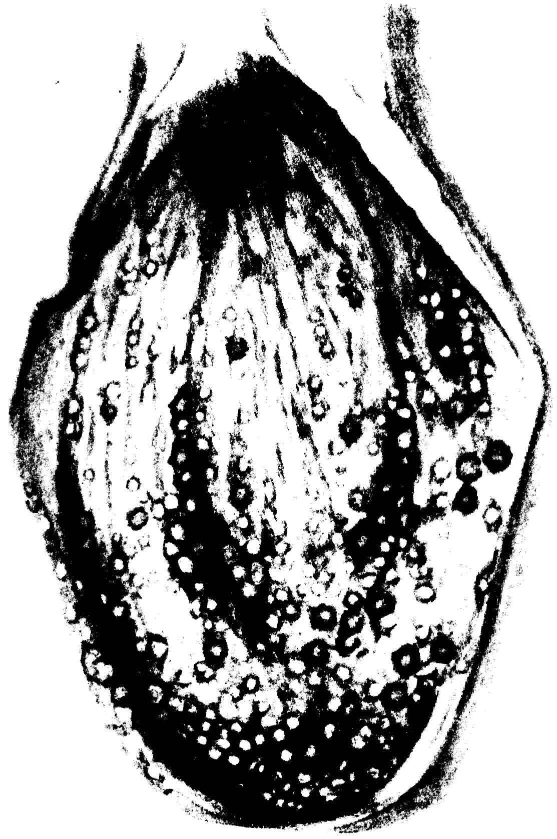

PLATE 21. RINDERPEST. GALL BLADDER. ACUTE INFLAMMATION OF THE MUCOUS MEMBRANE WITH SWELLING AND SUBSEQUENT NECROSIS OF THE FOLLICLES.

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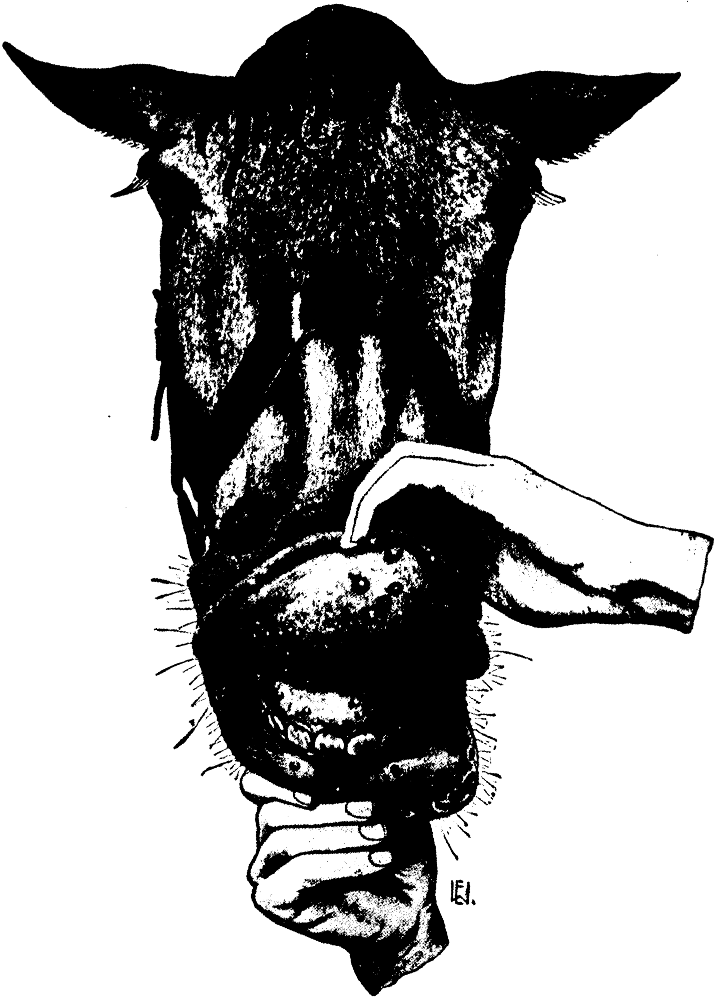

PLATE 22. INFECTIOUS STOMATITIS. NODULES, PUSTULES, AND ULCERATION.

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CHAPTER VIII

FILTERABLE VIRUS DISEASES OF ANIMALS

(CONTINUED)

MYXOMATOSIS OF RABBITS

Description.—This affection of rabbits was first described by G. Sanarelli(1) at the hygiene institute in Montevideo in 1898. It was again described, by Splendore(2) at Sao Paolo, in 1908. The affection is characterized by a purulent blepharoconjunctivitis which is rapidly followed by swelling of the head, also in the region of the rectum, the opening of the urethra, and the genital organs.

In 1905 Burgi(3) described an affection of rabbits which was similar to myxomatosis, but which he thought to be due to an infection with a strain of staphylococcus manifested by multiple suppurations. Burgi believed that this infection and its spread to enzoötic proportions could be traced to the transmission of an infection by fleas.

In 1910 Hermans(4) studied this condition and described it as a form of pseudotuberculosis in rabbits and identified it with syphilis in rabbits. (Hutyra and Marek.)

At autopsy animals dead of this disease show multiple gelatinous tumors in the subcutis. Hypertrophy of the lymph glands, orchitis, and enlargement of the spleen are also found.

The disease is transmitted artificially with ease, and in the absence of demonstrable microörganisms it is thought to be due to a filterable virus.

BIBLIOGRAPHY

1. SANARELLI, Centralbl. f. Bakt. **23** (1898) 865.
2. SPLENDORE, Centralbl. f. Bakt. **48** (1908) 300.
3. BURGI, Diss. Bern. (1905).
4. HERMANS, Ann. vét. (1910) 154.

NOVY'S FILTERABLE RAT VIRUS

In 1911 Novy(1) described a filterable virus which he discovered quite by accident while studying several strains of relapsing-fever spirochætes. It was his custom to maintain his strains of relapsing-fever spirochætes by the inoculation of blood from rat to rat.

All at once the rats began to die, usually within forty-eight hours. Upon investigation Novy could find no demonstrable bacteria present by either microscopic or cultural method. Filtration experiments disclosed the fact that a filter passer was the cause of the infection. The infectious agent not only passed through the ordinary Berkefeld filters but also easily passed through the finest filters, such as the Pasteur B and the Doulton. Furthermore, this agent was found capable of passing through collodion sacks and agar filters if not too thick. The virus contained in undiluted serum of the rat was capable of passing the finest filters. The virus was viable in 50 per cent glycerin for four to six months and ordinarily killed rats in from thirty-six to forty-eight hours. In high dilutions it required seventy-two to ninety-six hours to produce symptoms and death in the rat.

Novy (2) found that his rat virus was not centrifugable. When submitted to centrifugation at 8,000 revolutions per minute for twelve hours the virus was not thrown down. This was true even when the serum was diluted with a thousand times its volume of distilled water.

The blood of infected rats was found by Novy to be infective in extremely small doses. The virus was always fatal to rats even when quantities of blood were inoculated which represented only a fraction of a corpuscle. He states that "usually one billionth of a cubic centimeter will infect and we have obtained infections with one ten-billionth and even one-hundred billionth of a cubic centimeter. We can well imagine that in these high dilutions only a few organisms, possibly but one, are present."

It is doubtful if any of the filterable agents known to-day are as small as the one described by Novy. Unfortunately, after this virus had been propagated for several years the strain was accidentally lost. This is particularly unfortunate in that the study of this virus in relation to the respiration work described later by Novy would have added very important knowledge to the field of the filterable viruses.

BIBLIOGRAPHY

1. Novy, Physician and Surgeon **33** (1911) 243.
2. Novy, Trans. Clinical Soc. Univ. Michigan **2** (1911) 182.
3. Novy, Rep. Michigan Acad. Sci. **13** (1911) 31.

EPIZOÖTIC DISEASE OF GUINEA PIGS

We are indebted to Petrie and O'Brien (1) and to Sangiorgi (2) for what is known concerning this interesting disease of guinea pigs. Naturally occurring diseases in laboratory animals such as the guinea pig are of particular interest to the experimental biologist for obvious reasons. Particularly is this true where large institutions breed their own animals for experimental purposes. When disease enters the stock, experimental work is often delayed and results of experimental work are often difficult to interpret when epizoötics are present.

Petrie and O'Brien in 1910 investigated an epizoötic of guinea pigs that occurred in a stock of some five hundred guinea pigs at the Lister Institute, Chelsea. This disease killed 90 per cent of the stock guinea pigs. In searching for the causative agent of this disease these authors isolated a bacillus which closely resembles *Bacillus suipestifer* and *B. aertryck.* This organism was found to be present in the contents of the small intestine and was readily isolated from the fæces of sick animals at least three or four days before the death of the animals. The bacillus was also found in the liver and spleen but less frequently in the heart blood. Even when found in the blood there were comparatively few colonies and a marked septicæmia did not appear to exist. They were able to recover this bacillus in one instance from the bile but never from the urine.

The bacillus isolated from epizoötic disease of guinea pigs was found to be extremely pathogenic when injected subcutaneously into healthy guinea pigs. For guinea pigs weighing 250 grams, doses of 0.001 cubic centimeter of a broth culture invariably caused death within five days. Some animals succumbed following the injection of 0.000001 cubic centimeter but not always.

Rabbits and white rats were also found to be susceptible, the dose of infective material depending upon the route of injection. Inoculation per os did not always produce infection; in fact, it was difficult to infect animals by this avenue.

The local reaction following the subcutaneous injection of broth cultures of this bacillus consisted of an intense hæmorrhagic œdema and in older cases necrosis and abscess. In the natural disease there appear minute yellowish white nodules in the spleen and liver, but in animals dying from inoculation with the bacillus it was rare to find even minute spots in these organs. In the natural disease the intestines are congested as well as the adrenals and lungs.

Petrie and O'Brien have presented experimental evidence that the actual cause of this disease is a filter passer. While their results with filtrate experiments were not uniform, they found that sterile filtrates from organs of guinea pigs of the infected stock frequently killed. These authors offer the hypothesis that the disease is caused by a filterable virus, and that the bacillus isolated by them holds the same relation to this disease as *B. suipestifer* holds to hog cholera; namely, an associated bacterium and secondary invader.

The evidence presented for this concept may be briefly stated as follows: Due to the severity of this epizoötic it seems improbable that the bacillus is the actual cause of the disease when it was found so difficult to infect guinea pigs per os. The bacterium was almost constantly present in the fæces and there was ample chance for its spread by way of the intestinal tract, and yet experimentally this organism possessed low pathogenicity when given to animals with food. Furthermore, contact experiments failed, even though one animal badly infected with the bacterium was in the closest contact with normal animals. Lice from infected animals failed to transmit the disease to healthy animals. Finally Petrie and O'Brien observed that this bacillus is found in guinea pigs apart from an epizoötic and in apparently healthy animals. They conclude that the evidence suggests that the essential infecting agent in the epizoötic was a filter passer.

Mrowka(3) in his studies on fowl pest and guinea-pig pest virus demonstrated that the infecting agents were not damaged when precipitated from blood by tannin. This author found that these viruses were not part of the albumin fraction of the blood but were closely associated with the globulin fractions. According to his experiments it was impossible by repeated washings to dissociate the virus from the globulin fraction of the serum. Sangiorgi holds that these experiments do not prove definitely that the virus is associated with the globulins for the virus itself may be precipitated by such a reagent.

For the time being epizoötic of guinea pigs may be regarded as a filterable virus disease of unknown etiology.

BIBLIOGRAPHY

1. Petrie and O'Brien, Journ. Hyg. 10 (1910) 287–305.
2. Sangiorgi, Centralbl. f. Bakt. Parasitenkunde und Infektions-krankheiten, 1 Abt. 72, Orig. (1913–14) 70–73.
3. Mrowka, Centralbl. f. Bakt., etc. Arb. 1, Orig. (1913) 67.

SALIVARY-GLAND DISEASE OF GUINEA PIGS

Definition.—According to Cole and Kuttner(1) the occurrence of intranuclear inclusions in the salivary glands of guinea pigs is due to an infection with a filterable virus. Experimentally, when an emulsion of the infected submaxillary glands of full-grown guinea pigs is injected into the brain of young guinea pigs, there result fever and symptoms of cerebral irritation. At autopsy such animals show a subacute meningitis and numerous cellular inclusions in the exudate.

History.—In 1920 Leila Jackson (2) first described the presence in the salivary glands of guinea pigs of an intracellular protozoan parasite. Jackson examined the salivary glands of forty-eight guinea pigs, eighteen of which were tuberculous. Of this number twenty-six animals were infected and the infection occurred in about the same proportion in the tuberculous guinea pigs and in the presumably normal animals. According to this author the protozoan most often appears in the cells of the ducts of the salivary gland as an encysted, round or oval structure. These structures were found particularly in the serous portions of the glands, but in severely infected animals they were also found in the mucous portions. Jackson believed that she was dealing with the vegetative cycle of an intracellular protozoan, perhaps coccidium, the sexual cycle of which occurs in some other location. The small round bodies that she described in the peripheral zone of the parasite were thought to represent merozoites.

In 1926 Cole and Kuttner studied the salivary glands of seventy-five guinea pigs over six months of age. They found that 63, or 84 per cent, were positive for these structures when stained by eosin and methylene blue. They identified these cells as swollen epithelial cells, the nuclei of which contain a mass of granular material that is definitely acidophilic. By transmission experiments they were able to conclude that they were dealing with a virus disease.

The virus of salivary-gland disease of guinea pigs.—Cole and Kuttner state that the infective agent in this disease is destroyed by heating at 54° C. for one hour, and that preservation in 50 per cent glycerol for eleven days does not destroy its activity. According to these authors the virus becomes inactive after twenty-eight days in 50 per cent glycerol. They further demonstrated that the infective agent is filterable through Berkefeld N filters which are impermeable to bacteria.

Incubation period.—When emulsions of infected salivary glands taken from full-grown guinea pigs were injected into the brains of young noninfected guinea pigs there was a definite rise in temperature within forty-eight hours to 105 or 106° F. On the third day the inoculated animals were definitely sick. According to the work of Cole and Kuttner then, the incubation period in experimental infections with this virus may be regarded as very short.

Symptoms.—In normal animals harboring this virus in the salivary glands there are apparently no constitutional symptoms. The disease manifests itself only by the peculiar histologic findings at the site of the infection. In the young guinea pigs inoculated intracerebrally by Cole and Kuttner with infected salivary-gland material, definite symptoms were obtained. Following the rise in temperature which results forty-eight hours after injection, the animals appear sick, the hair is raised, and the animals do not move in their cages. On the fourth day the animals develop slight tremors and convulsions. By the fifth day inoculated animals are very ill, unable to rise, and soon succumb to the infection. Young guinea pigs that received intratesticular inoculations of virus developed an elevation in temperature but were normal otherwise. Injections of infective material into the tongues of young animals were followed by no symptoms. An inoculation made into the lung of one guinea pig resulted in a rise in temperature on the seventh day, at which time the animal was sacrificed for histologic study.

Animals susceptible to the virus of guinea-pig salivary-gland disease.—At present only guinea pigs are known to be susceptible to this virus. Cole and Kuttner found that rabbits, rats, and kittens remained well after inoculation with infective material and no histologic lesions could be demonstrated in these animals.

Immunity.—At present no data are available on this phase of the disease. Cole and Kuttner found that only three out of forty-three young guinea pigs, mostly under one month of age, were infected. Later in life nearly all guinea pigs become infected.

Pathology.—It has been mentioned that what Jackson took to represent protozoan parasites, Cole and Kuttner believe to be swollen epithelial cells with nuclei having the same characters as the nuclei of the atypical cells in the lesion of herpes simplex. The altered cells may be from twice their normal size up to 40 microns in diameter. They are found in both the serous and mucous portions of the gland, but predominately in the serous portion. They lie on the basal membrane or within the lumen of the duct. In some cases large numbers of ducts are involved while in others only one or two ducts may be affected. There is a cellular reaction about the ducts containing these cells which consists of mononuclear cells, lymphocytes, and large cells with vesicular nuclei (Cole and Kuttner). The cytoplasm of the cell stains light blue, the nuclear membrane stains deeply with basic dye, and near the center of the nucleus is a mass that stains red. This mass may occupy one-fourth of the nuclear space or fill it entirely except for a narrow halo between it and the membrane. This clear area may contain irregular masses which stain deeply with the basic dye. Also, radiating bands which stain with basic dye may be found extending from the inner surface of the membrane to the central mass. In the main these structures resemble closely the structures found in herpes simplex. Such structures could not be demonstrated by Cole and Kuttner in the salivary glands taken from rabbits, rats, mice, dogs, or cats. Further study will be necessary in order to determine the exact nature of these bodies. From the evidence presented by Cole and Kuttner it appears that in this disease of guinea pigs we have still another condition in which intranuclear inclusions are associated with an infection with a filterable virus. In the transmission experiments of these authors typical structures were demonstrated in the exudate of guinea pigs infected intracerebrally, in 75 per cent of the animals receiving intratesticular inoculations, in the tongues of animals inoculated at this site, and in the lungs of one out of three animals receiving direct injections into this tissue. Efforts to transmit the infection indefinitely in series failed, although it was possible to reproduce the lesions through two animals in series and in one experiment through three animals in series. No explanation has been offered for this failure.

BIBLIOGRAPHY

1. Cole and Kuttner, Journ. Exp. Med. **44** (1026) 855–873.
2. Jackson, Journ. Infec. Dis. **26** (1920) 347–351.

For other references see the chapter on Intracellular Inclusions.

GUINEA-PIG PARALYSIS

Like epizoötic disease of guinea pigs this disease of guinea pigs is of particular interest to the experimental biologist. Guinea-pig paralysis is a good example of a naturally occurring disease in laboratory animals, the symptoms of which were first thought by Römer to be part of the picture of an experimental disease he was studying. As we pointed out in the description of epizoötic disease of guinea pigs, it is essential for the biologist to become informed regarding the naturally occurring diseases in experimental animals in order that he can evaluate and interpret experimental results. This disease and its discovery is a case in point.

In 1910 Römer(1) first called attention to a paralytic condition in guinea pigs which he believed at that time was related to the experimental diseases he was studying in this animal. He pointed out that the disease was not extraordinarily rare in guinea pigs. In 1911 Römer(2, 3) studied this disease further and concluded that the affection is a disease sui generis. In the artificially induced infection the disease is characterized by an initial rise in temperature followed by a slight loss in weight. The incubation period ranges from nine to twenty-three days and there are no further symptoms following the intracerebral inoculation for some time. The initial rise in temperature and loss in weight are regarded by Römer as incidental to the surgical shock following intracerebral inoculation. After an incubation period of nine to twenty-three days the disease begins with an elevation in temperature followed in one or two days by a loss in weight and nerve manifestations. The latter consist of daily increasing hypotony of the muscles, particularly of the hind legs. The comparison of the muscles of the sick animals with those of healthy animals is very striking. In some cases the first symptoms are related to the bladder, and incontinence of urine is observed. According to Römer complete paralysis of the bladder may ensue. Guinea pigs with this disease when dropped or let loose on a table fall upon their sides, while normal animals always fall upon all four feet. The paralysis of the hind legs gradually increases until complete paralysis exists. When the bladder symptoms are absent Römer states that the disease simulates closely the Heine-Medin, poliomyelitis, sickness of man.

The duration of the disease varies considerably. Death may result within two or three days after the onset of symptoms. Usually the disease persists for eight or ten days, and in one case the animal did not die for four weeks after the onset of symptoms. The mortality is 100 per cent.

The virus of guinea-pig paralysis.—Guinea-pig paralysis is caused by a filterable virus. All attempts to demonstrate bacteria or protozoans in stained smears from nerve tissue have failed. Culture methods have all been unsuccessful with this virus. The virus is filterable through Berkefeld filters, the filtrate being capable of inducing the infection in healthy guinea pigs. There is no evidence that any other animal is susceptible to the virus of guinea-pig paralysis. Römer was able to transmit the disease in series from guinea pig to guinea pig by brain and spinal-cord emulsions when inoculated intracerebrally. The animals inoculated with 5 per cent emulsion of the material exhibited the same incubation period as those inoculated with one hundred times diluted material. A 1 : 10,000 dilution of the infective material failed to induce the disease.

Animals inoculated with Berkefeld-filtered material came down with the disease within the same incubation period as those inoculated with unfiltered material. The virus was recovered from these animals. The filters employed by Römer were tested with *B. prodigiosus*. The virus was found to be resistant to glycerin. Material kept for ten days in 50 per cent glycerol was found to be potent. Within fifty-six days the virus is destroyed by 50 per cent glycerin.

The virus of guinea-pig paralysis is present uniformly in the tissue of the central nervous system and in some cases was found by Römer in the spleen, liver, and lymph glands. The virus was never present in the kidneys, lungs, bile, or urine. While the disease always follows intracerebral inoculation, results were for the most part unsatisfactory when infective material was inoculated subcutaneously, intravenously, intraperitoneally, or intraneurally.

The anatomical changes in the central nervous system are characterized by a meningo-myelo-encephalitis. The infiltrating cells are predominately of the lymphocytic type. In this respect there is a close analogy between this disease in guinea pigs and encephalitis and poliomyelitis in man.

Herpes infection of the central nervous system in laboratory animals occurring as a natural infection has not been reported. It is well known, however, that an encephalitic process can be induced in guinea pigs, rats, and rabbits with various strains of herpes virus. Also it is now recognized that spontaneous encephalitis in rabbits is fairly common, although herpes is in no way connected with this affection. Encephalitis also occurs in horses and sheep. Guinea-pig paralysis in Römer's experience occurs only as sporadic cases and never in epidemic form. One wonders if there is a possibility of this filterable virus being related to the herpes group. The fact that other laboratory animals were not susceptible to Römer's virus is against this view. However, when opportunity presents itself cross-immunity experiments with the guinea-pig-paralysis virus and the herpes viruses should be performed.

The evidence all supports the view that guinea-pig paralysis is caused by a filterable virus, and in view of its high infectivity for guinea pigs this virus should receive further attention. It appears to be ideal material for the study of some of the more fundamental problems dealing with the study of the filterable group of infectious agents.

BIBLIOGRAPHY

1. RÖMER, Münch. med. Woch. (1910) No. 51.
2. RÖMER, Deut. med. Woch. 37 (1911) 1209.
3. RÖMER, Centralbl. f. Bak., Beilage, Abt. 1, Ref. (1911) 50, 30.

NOGUCHI'S FILTERABLE VIRUS FROM DERMACENTOR ANDERSONI

Description.—In 1926 Noguchi (1) described a filter-passing virus which he isolated from one lot of ticks collected in Saw Tooth Canyon, Montana. This virus proved to be infectious for guinea pigs and one monkey (*Macacus rhesus*), though one rabbit inoculated remained negative.

In Noguchi's experiments ticks were allowed to feed upon normal guinea pigs. Ticks 1, 3, and 4 were found gorged with blood, tick 2 died. The ticks were eviscerated, the viscera were individually suspended in salt solution, and 1 cubic centimeter of each suspension was injected into a normal guinea pig. The suspensions of ticks 1 and 2 induced spotted fever, that of tick 4 produced no reaction. The suspension of tick 3 induced a fever similar to spotted fever, but this did not confer immunity to the spotted-fever virus. It was from tick 3 that the filter-passing virus was obtained.

Noguchi found that he could cultivate this filterable virus upon semisolid media for at least seven generations. The virus is aërobic, grows at 26 and at 37° C., and does not grow in ordinary broth or on slant agar. It produces a slight turbidity when grown in serum media containing carbohydrates. The blood and spleen of infected animals contain the virus. The virus is filterable through Berkefeld N filters.

According to Noguchi the virus has been transmitted from infected guinea pigs to ticks and in one instance by a tick feeding from an infected tick to a guinea pig. Infection of guinea pigs with this virus affords no protection against the virus of spotted fever or vice versa.

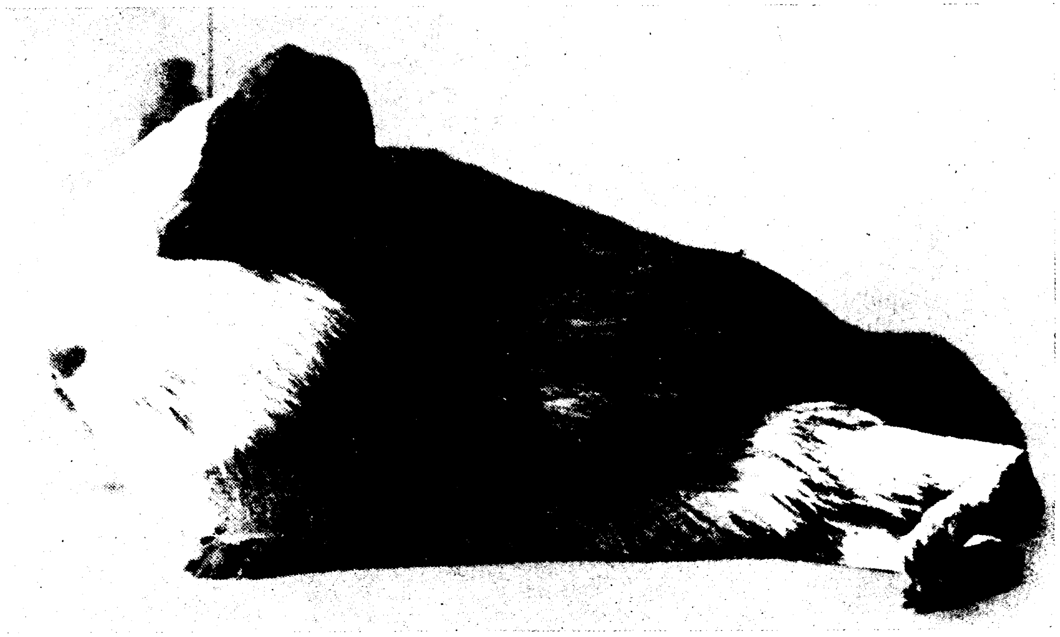

PLATE 23. GUINEA-PIG PARALYSIS.

The chief symptoms in guinea pigs consist of a continuous high fever of 104.5 to 106.5° F., and enlargement of the spleen.

This virus should be of importance to students of the filterable viruses, since it appears to be one of the few that can be cultivated artificially.

Reference 1. Noguchi, Journ. Exp. Med. 44 (1926) 1–10.

ILLUSTRATION

Plate 23. Guinea-pig paralysis. (After Römer.)

CHAPTER IX

OTHER POSSIBLE FILTERABLE VIRUS DISEASES OF MAN

COMMON COLDS: ACUTE CORYZAS

Under the heading of common colds we include a group of acute infections (exudative and catarrhal) of the nose, throat, larynx, trachea, and larger bronchi. In some cases the sinuses are involved. Epidemics of "colds" are common, and the infection appears to spread with great ease. The infection may localize in one membrane such as the nose, rhinitis, or in the pharynx, pharyngitis, or two or more membranes may be involved. In some cases the sinuses, the naso-pharynx, throat, trachea, and larger bronchi may all be affected. "Common colds" should be regarded as an acute infectious disease and not merely as congestion of the respiratory membranes produced by drafts of cold air, changes in temperature, etc. Under normal circumstances the ciliated epithelium of the respiratory tract prevents to a large degree the entrance of bacteria from without. However, this mechanism is not perfect and untold numbers of microbes enter the respiratory tract. Also it is now known that the lacrymal and nasal secretions exert a mild germicidal action and this is likewise true for the saliva. There is some evidence that the secretions from other mucous membranes of the respiratory tract possess bacteriocidal properties. The mechanical and chemical nature of the mucous membranes of the respiratory tract then tends to prevent infection under normal conditions. Why then are colds so prevalent? It is estimated that about 10 per cent of human beings are at all times affected by "common colds" of greater or lesser degree. Undoubtedly there are many predisposing causes to the development of common colds, but the chief reason common colds are so prevalent is the fact that they are due to acute infections caused by one or more specific infectious agents.

The "common cold" has attracted more attention in recent years than ever before. The medical profession and the public at large have begun to realize the importance of this disease, especially in its relation to epidemic influenza and pneumonia and its economic importance to the nation in the enormous loss of working hours which results from these infections.

Bacteriologists have long been concerned with the problem of the common cold, and many attempts have been made to isolate the causative organism. At various times such microbes as pneumococci, influenza bacillus, streptococci (both hæmolytic and viridans), staphylococci, *Micrococcus catarrhalis,* and diphtheroids have been cultured from the respiratory tract of cases of common colds. Of the relation of any of these bacterial forms to colds little is known. So far there is no direct evidence that any of these bacteria should be incriminated as the single etiological factor of these infections.

Hüter(1) in 1873 first described a micrococcus as the cause of common colds. In 1888 Hajek(2) reported the presence of a large diplococcus, *Diplococcus coryzæ,* found in the early stages of acute colds. Paulsen(3) in 1890 examined a number of cases of common colds and found various cocci and bacilli in the noses and throats of these patients. Cautley(4) a few years later discovered the presence of a diphtheroid bacillus in seven of eight cases of colds of various types. He believed this organism to be the cause of acute colds and designated it *B. coryzæ segmentosus.* Benham(5) quotes Gordon as having found Cautley's bacillus in seven cases of colds. With this organism Gordon was able to produce illness in guinea pigs from which they recovered after a few days. Benham also quotes White as having found Cautley's bacillus in seventeen of twenty-one cases, although inoculation experiments on guinea pigs, rabbits, and monkeys were negative.

In 1906 Benham found a diphtheroid organism, which he believed to be identical with Cautley's organism, in twenty of twenty-one cases of colds. He named this organism *Bacillus septus. Micrococcus catarrhalis* was also present in several of Benham's cases. In 1902 Gohn and Pfeiffer(6) found *Microccus catarrhalis* in eighty-one of one hundred forty cases of infections of the respiratory tract. These authors regarded the presence of this organism in respiratory infections as a saprophyte which, under favorable conditions, gives rise to acute infections. Benzancon and de Jong(7) during an epidemic of influenza in Paris in 1905 found various organisms present in the respiratory tract. Among these were *Micrococcus catarrhalis, Micrococcus paratetragenus, Pneumococcus,* Friedlander's bacillus, staphylococcus, streptococcus, and diphtheroids. During the same year Dunn and Gordon(8) pointed out the etiological importance of *M. catarrhalis* in an epidemic in Hertfordshire, England, while Allen(9) during the following year isolated *M. catarrhalis* from several cases of common colds in the same country. Neumann(10) in 1902, as a result of his extensive studies on the etiology of common colds, concluded that diptheroids and pneumococci, and perhaps other organisms, could produce colds. This investigator found *Micrococcus pyogenes albus* present in 86 to 90 per cent, diphtheroids in 98 per cent. He states that seventy-eight strains of diphtheroids isolated by him were found to be nonpathogenic when inoculated into guinea pigs, although pathogenic strains of this organism were found in infected cases. Both Lingelsheim(11) and Barnes(12) have shown that streptococci may cause infections of the nose, pharynx, and tonsils. The streptococcus has, of course, long been established as an etiological agent in epidemic sore throat. Mathers(13) in 1917 and Floyd(14) in 1920 described streptococci and staphylococci in relation to epidemics of acute respiratory infections occurring in Chicago and Boston.

In 1920 in the Boylston prize essay, Mudd, Grant, and Goldman(15) have reviewed the entire literature on the acute respiratory infections and after a careful analysis of the various bacteriologic findings state:

> Since the early days of bacteriology, attempt has been made by the several proponents and opponents of the infectious theory to refer the "common cold" on the one hand to the action of a specific microörganism, and on the other hand to various environmental and constitutional causes, such as exposure to changes of temperature, the "lithemic diathesis" and what not. Although perhaps laudable as philosophic ideals, such efforts to explain the many phenomena involved by a single cause are less deserving scientifically, and have met with just failure. The common cold is, as a matter of fact, in most instances the result of a local infection, but there are many types of cold and many infectious agents responsible for them, and the effect of various constitutional and environmental factors in determining infection is often of great importance. Furthermore there are many acute inflammations of the upper respiratory tract not primarily due to the local action of microörganisms, but rather to local expression of chemical or mechanical irritation, of thermal trauma, of nervous reflexes, of drug intoxications, of constitutional disease, or anaphylaxis.

In 1913 Tunnicliff(16) investigated eighty-two cases of common colds in Chicago, and from 92 per cent isolated a bacillus to which she gave the name *B. rhinitis.* Also this investigator found the same organism in 90 per cent of twenty cases of chronic colds, rhinitis. In normal throats she was able to produce a slight inflammation with *B. rhinitis,* and the organism was recovered in most cases from the artificially infected throats eighteen to twenty hours after inoculation and in a few cases two or three days later. Tunnicliff vaccinated two volunteers with this organism, and from variations in the opsonic index she believed that there was evidence that this organism is the cause of common colds.

Kruse(17) in 1914 filtered the nasal secretion from a case having acute coryza and then placed a few drops of the filtrate upon the nasal mucous membranes of twelve volunteers. In four of these cases acute colds developed. The period of incubation in the positive "takes" ranged between one and four days. In this experiment the infected nasal secretions were diluted fifteen times with salt solution. In another experiment Kruse diluted the infected nasal secretions from an acute coryza patient with twenty times its volume before filtration. In the test experiment with this filtrate thirty-six students were inoculated, and 42 per cent developed acute symptoms of coryza within one to four days.

The experiments of Kruse were repeated by Foster(18) in 1915. This investigator confirmed the presence of a filterable virus in the nasal secretions of cases of acute colds. Material taken from a case of acute coryza, diluted and passed through Berkefeld N filters, when inoculated into nasal cavities of ten soldiers produced symptoms within eight to thirty hours in nine instances. In seven of Foster's cases the symptoms of acute coryza were definite and clear cut; two reacted questionably, while one case exhibited no symptoms. Foster states:

> The initial symptoms, as a rule, were dryness of the nose and throat and attacks of sneezing, dull frontal headache and a sensation of pain or fullness over the frontal sinuses. Several of the men complained of alternate sensations of chilliness and flushing. There was a copious rhinorrhea, usually on the second day, in a majority of the cases. Six of the subjects exhibited slight rise in temperature—99.2–100° F. and in these cases the pulse was accelerated. Tinnitus aurium or slight impairment of hearing was recorded in four instances; sore throat in 5; cough 5; and aching pains in the back or extremities in 4. One of the men complained of parotid tenderness and pain on moving the jaw; in another case a marked crop of herpes labialis was noted. The duration of the symptoms varied 3 to 6 days—usually 5.

Foster was unable to obtain growth of any organisms from early cultures of his filtrates, but under the dark-field microscope he noted myriads of extremely active, minute bodies occurring singly, in pairs, and in masses varying in size. In older cultures, fourteen days, of his material Foster found minute coccoid bodies which varied in size from those barely visible to those larger than staphylococci. The larger bodies often showed smaller bodies adhering to them. This microbe stained with Giemsa's but not well with ordinary laboratory stains. The predominating type noted by Foster was a small globoid body measuring 0.2 to 0.3 micron in diameter. In conclusion Foster states:

> It becomes evident at once that this microörganism differs markedly from any known organism with the possible exception of the "globoid bodies" described by Flexner and Noguchi,(19) and believed by them to be the causative factor in poliomyelitis. An extremely pleomorphic streptococcus, the minute forms of which bear some resemblance to this microörganism has recently been described in connection with poliolyelitis, also, by Mathers,(20) Rosenow, Towne and Wheeler,(21) and Nuzum and Herzog.(22)

Later Foster compared his microbe isolated from cases of common colds with the organism described by Flexner and Noguchi for poliomyelitis and also with the streptococcus described by Rosenow, Towne, and Wheeler. His examination showed that the organism isolated from common colds differs from the poliomyelitis virus in that the former is subject to many variations in size and the larger forms show evidence of budding, neither of which is true for the Flexner-Noguchi virus of poliomyelitis. Foster found that his organism showed little tendency to chain formation; and the pleomorphic forms, common to the streptococcus, were absent. Foster was also able to demonstrate that these cultures were filterable through Berkefeld N filters, and that filtrates of the cultivated organism in the second generation are infective when tested upon volunteers.

The question naturally arises as to whether this microbe might not exist in symbiosis with the true ultravirus of common colds. No instance of such a phenomenon, however, is known, with the possible exception of hog cholera. Again the organism described by Foster might be a stage in the life cycle of an ultravirus and modern investigations upon the filterable forms of bacteria (tubercle bacillus, streptococci, etc.; see Chapter XVI) lend support to this concept. In this connection Foster recalls the work of Hurt, Lakin, and Benians in which these authors suggest that epidemic meningitis may be due to an ultravirus while the meningococcus found in the spinal fluid of these cases represents "a late phase in the life history of an unidentified microörganism that is the true infective organism." These authors demonstrated that it was possible to obtain a pathogenic virus from the spinal fluid of acute cases of epidemic meningitis after the spinal fluid had been passed through a Chamberland F' filter.

In support of this theory Foster points out that the microbe isolated by him from cases of common colds shows a considerable variation in size, and, further, that from seven to fourteen days are needed in order to obtain growth of the virus. The virus is cultivated under anaërobic conditions. In conclusion Foster says, "Although conclusive proof of its nature has not been adduced, the experiments suggest that the microörganism described bears a definite relation to the true infective agent of common colds."

Mudd, Grant, and Goldman in the Boylston prize essay, after a careful survey of all the important investigations which have been made on the etiology of the common cold, further state:

> A filterable virus seems without question to be the causative agent in the coryza of one fairly well defined type.—A common and fairly well defined clinical entity, and acute coryza, exists, probably with the filterable virus of Kruse and Foster as its causative agent. This affection is readily communicable and probably does not depend to any great extent upon the action of exciting factors in depressing the resistance of the subject. (2) A heterogenous group of pure and mixed infections of the nose, pharynx and tonsils exists with various clinical pictures—some closely approaching that of Foster, others mere circumscribed inflammations—and with any one of a considerable number of bacteria capable, under appropriate circumstances, of acting as causative agents.

Of the bacterial forms which have been described by various investigators it appears that the pneumococcus, the streptococcus, *B. diphtheriæ*, *B. rhinitis*, Pfeiffer's bacillus, and Friedländer's bacillus have the best claims to etiological import in relation to the acute respiratory infections.

While it is well recognized then that the common cold is to be regarded as an infectious disease, authorities are also agreed that there are a number of predisposing factors in the development of such an infection. Furthermore, there may be symptoms of coryza which are not related to infection but which may be caused by one or more of these factors. It has long been thought by the layman and by physicians that drafts of cold air may cause colds. Chilling of the body surface, particularly one portion of the body surface, is now thought to be a predisposing cause in the development of colds. In fact the general statement may be made that anything which lowers the resistance of the host may predispose to infections. Practically all texts are agreed on this thesis. It follows then that exposure to cold drafts contributes to the development of colds. Mudd, Grant, and Goldman have shown that the chilling of the body surface causes reflex vasoconstriction and ischæmia of the mucous membranes of the nasal cavity, nasopharynx, palate, oropharynx, and palatine tonsils. These authors believe that ischæmia may be the means of lowering the local resistance to infection. Rosenau(23) states:

> Chilling causes vasomotor contraction of the capillaries of the skin, which is doubtless designed to conserve body temperature; coincidently there is turgidity of the erectile tissue of the mucous membrane of the turbinates, which is probably a defensive action. This congestion partly closes the nose and causes snuffing and increased secretion, which is ordinarily called a cold. A great variety of mechanical, chemical and even psychic stimuli will produce congestion of the cavernous tissue over the turbinate; in fact, the mucous membrane of the nose may become very sensitive, even hypersusceptible. Anaphylactic reactions to pollen and proteins are common manifestations of the nasal mucosa.

Vaughan(24) states in this connection:

> In our opinion, the acute coryzas are most rationally explained on the ground that they are due to protein sensitization. They may be caused by any protein, living or dead, organized or unorganized, particulate or in solution. Omitting from consideration Foster's theoretical globoid bodies, suspected in his subcultures, his experiments and those of Kruse are most easily explained on the ground of protein sensitization. These experiments are practically duplications of the opthalmic tuberculin test. A minute trace of tuberculin dropped into the eye of a patient in the early stages of tuberculosis causes an exudative inflammatory action. In all probability, Foster's soldiers had been previously sensitized to the same protein which existed in his nasal secretions and in those of his laboratory assistant. If this be true, it could not be otherwise than that an inflammatory reaction would result within a few hours after the application of this protein to the nasal mucous membrane of these soldiers.

When writing the above Vaughan was not informed of the later work of Foster in cultivating the microörganism with which he was able to produce colds in volunteers. Perhaps the main thesis of his theory of the origin of colds he would have stated notwithstanding. However, in view of Foster's work, confirming and extending as it does the experiments of Kruse, and further in view of the recent studies of Olitsky and Gates(25) on a filterable agent which they believe to be the cause of influenza, science is not ready to accept Vaughan's theory of sensitization for common colds. Neither, for that matter, are we willing, without more conclusive data, ready to accept the filterable virus origin of colds and influenza. However, in the broad interests of science we must give these theories and experiments serious consideration.

In view of all we have been able to learn by experimental work and through observation, we are inclined to agree with Mudd, Grant, and Goldman that a clinical entity exists in the form of the infectious cold. Similarly it is well known that coryza is common in some people who become sensitized to various proteins. Consequently a classification of colds might be suggested as follows:

1. Common colds due to infectious agents.
    (*a*) Various pathogenic bacteria.
    (*b*) Filterable viruses.
2. Colds due to local sensitization to various proteins.

To these groups should be added those predisposing causes which, in and of themselves, are not the causes of colds but contribute to the development of colds by creating favorable conditions for infection by microörganisms or sensitization to various proteins other than infectious agents. Under this heading we should include: Drafts of cold air which produce a chilling of the body surface; chemical or mechanical irritation; thermal trauma; nervous reflexes; drug intoxications; constitutional diseases; and psychic influences. In general any factor which may lower the resistance of the host to infection or sensitization may be regarded as a predisposing cause in the development of common colds.

While the subject of the common cold is now recognized to be one of the most important problems from the standpoint of health and disease of the race, there remains much to be done in careful investigation of its cause or causes. We are inclined to feel that Foster and Kruse have given us convincing evidence that at least one type of cold may be due to a filterable virus and that future investigative work should be done along these lines to enable us better to understand the nature of these agents and the mechanism by which infection takes place.

BIBLIOGRAPHY

1. HÜTER (1873). Quoted by Benham. (See 5.)
2. HAJEK, Berl. klin. Wchnschr. 33 (1888), 659.
3. PAULSEN, Centrlbl. f. Bakt. 8 (1890), 344.
4. CAUTLEY, Report of the Local Govt. Board, Supplement, Great Britain (1894–95) 455.
5. BENHAM, Brit. Med. Journ. 1 (1906) 1023.
6. GOHN and PFEIFFER, Ztschr. f. klin. Med. 44 (1902) 262.
7. BEZANCON and DE JONG, Bull. et mem. Soc. méd. d. hop. de Paris 22 (1905) 165.
8. DUNN and GORDON, Brit. Med. Journ. 2 (1905) 421.

9. Allen, Brit. Med. Journ. 1 (1906) 1131.
10. Neumann, Ztschr. f. Hyg. 40 (1902) 33.
11. Lingelsheim, Kolle and Wassermann's Handb. d. Pathogenen Mikroorgan. 4 (1912) 481.
12. Barnes, The Tonsils. C. V. Mosby Co., St. Louis (1914).
13. Mathers, Journ. Infect. Dis. 30 (1917) 1.
14. Floyd, Boston Med. and Surg. Journ. 182 (1920) 389.
15. Mudd, Grant, and Goldman, Journ. Lab. and Clin. Med. 6 (1920–21) 175, 253, 322 (lit.).
16. Tunnicliff, Journ. Infect. Dis. 13 (1913) 283; 16 (1915) 493.
17. Kruse, München med. Wchnschr. 61 (1914) 1547.
18. Foster, Journ. Am. Med. Assoc. 66 (1916) 1180; Journ. Infect. Dis. 21 (1917) 451.
19. Flexner and Noguchi, Journ. Exp. Med. 18 (1913) 461.
20. Mathers, Journ. Am. Med. Assoc. 67 (1916) 1019.
21. Rosenow, Towne, and Wheeler, Journ. Am. Med. Assoc. 67 (1916) 1202.
22. Nuzum and Herzog, Journ. Am. Med. Assoc. 67 (1916) 1205.
23. Rosenau, Preventive Medicine and Hygiene. Appleton & Co., New York and London (1927).
24. Vaughan, Epidemiology and Public Health. C. V. Mosby & Co., St. Louis (1922).
25. Olitsky and Gates, Journ. Am. Med. Assoc. 76 (1921) 740.

INFLUENZA

Epidemic influenza; La grippe; Grip

Definition.—Influenza is an acute, highly communicable, febrile disease, characterized by catarrh of the respiratory tract and in some cases of the alimentary tract, by pains in the head and musculature, by its tendency to bronchial and pneumonic complications, and by its occurrence in epidemic and pandemic form. The disease is also notable for its sudden onset and extreme prostration. The cause of influenza has not been definitely determined, but it is thought to be due to a minute filterable virus, *Bacterium pneumosintes,* described by Olitsky and Gates.(1)

History.—Vaughan(2) says, "What appears to be the first recorded instance of an influenza epidemic is referred to in Livy in 412 B. C." While according to this author these early reports are not authentic, the disease appears to have been influenza because of its sudden onset and wide distribution. Vaughan quotes Hirsch as looking upon the year 1173 as witnessing the first epidemic of which authentic reports are available. Influenza during that year spread over Italy, Germany, and England. This author states that the first great pandemic of the disease occurred in 1510. At this time influenza spread from Malta to Sicily, thence into Spain, Italy, Hungary, Germany, France, and England. The symptoms of the disease consisted of fever, cough, hoarseness, headache, anorexia, insomnia, pains in the stomach, kidneys, and legs. Symptomatically then this pandemic appears to have been due to influenza. From this time on history records numerous pandemics of the disease. Great pandemics are known to have occured in 1590, 1732, 1781, 1830, 1833, 1836, 1847, 1850–51, 1855, 1857–58, 1874–75, and 1889. In North America epidemics have occured since 1557. Vaughan records the various epidemics as follows: 1557, 1580, 1647, 1732, 1737, 1760, 1780, 1789, 1805, 1824, 1830, 1836, 1843, 1850, 1860, 1863, 1873, 1874, 1879, 1889, 1891, 1916, 1918. The last great pandemic occurred in 1918–19 and again in the winter of 1919–20. The disease entered America in September of 1918. Thousands of men were mobilized in the army camps and the disease swept through these hordes of men like a tidal wave, leaving great numbers of dead in its wake. In this epidemic the disease had its highest mortality in the age group of twenty to thirty-five years (though the disease was by no means confined to this group), and for that reason greater mortality resulted in the army than in civilian life. During this year the disease spread all over the world to pandemic proportions and is considered the most severe outbreak of influenza in the history of all time. According to Vaughan (Warren) the 1918 pandemic had its origin in the United States and the virulence of the organism was enhanced by passage to and from Europe. The common complication of the disease in this epidemic was pneumonia. The pneumonia rate was somewhat higher among colored troops than among white troops as was also the death rate. Out of 1,439,000 men under arms in the United States, Vaughan states that 338,343 had influenza, or 22.6 per cent. The death rate among these troops during this epidemic was 1.56 per cent. In the American Expeditionary Forces abroad there were 1,745,000 men under arms and 77,828 of these had influenza. The death rate in this group was only 0.48 per cent. This has been explained by the fact that the soldiers in Europe were seasoned men, who came from camps where influenza had been present and so had acquired some immunity to the disease. While the mortality in civilian life during this epidemic was lower than in the army, it was greater in the cities than in rural communities. Undoubtedly the crowding of human beings contributes to the great spread of the disease. Influenza occurs in succession and does not attack a people en masse. It is spread from person to person through coughing, sneezing, and by droplet infection from the mouth, nose, and throat of infected persons. Carriers of the virus of influenza may transmit the disease to healthy persons though they are themselves unaffected by it. Dust may transmit the disease from infected persons to healthy persons, but for the most part the disease is disseminated from person to person. A comprehensive analysis of the last great pandemic of influenza is given by Vaughan and for a detailed analysis the reader is referred to his work.

Distribution.—Influenza may occur in pandemic form and exist practically all over the world wherever man, its natural host, is found. Small epidemics are undoubtedly in existence in some parts of the world at all times. Periodically the disease spreads to pandemic proportions and must be explained either by a diminution of the normal resistance or immunity of the host, or by an increase in the virulence of the virus or perhaps a combination of both.

The virus of influenza.—In 1892 Pfeiffer(3) discovered the influenza bacillus in thirty-one cases of clinical influenza. The bacillus was present in smears from the pharynx and sputum. This organism is a small, Gram-negative, nonmotile, hæmoglobinophilic bacillus which grows aërobically at 37° C. on blood agar. Various investigators have found this organism almost constantly in the throats of influenza cases. It is present in about 30 per cent of normal throats and also occurs in cases of measles and whooping cough and in other infectious diseases. Pfeiffer claims to have produced influenza in a few instances in monkeys and atypical forms of the disease in rabbits. It is interesting to note that during the 1918 epidemic various organisms were reported from the different army camps in the United States as predominating in the cases of influenza in those particular localities. In some instances staphylococci predominated, in other cases it was a streptococcus or a pneumococcus, while in others the Pfeiffer bacillus was the commonest organism found. All of these observations have led to the opinion that the influenza bacillus of Pfeiffer may be only a secondary invader, and while not the actual cause of influenza it may become exceedingly virulent and contribute to pneumonia, the most severe complication of the disease. Even now opinion remains divided as to the rôle the influenza bacillus plays in epidemic influenza. Some authorities still believe in its etiologic significance, and the question remains unsettled. After the discovery by Pfeiffer of the influenza bacillus this investigator found in three cases of bronchopneumonia following diphtheria in children a similar, although somewhat larger, bacillus which he designated pseudoinfluenza bacillus. Whether or not this organism represents still another strain of the influenza bacillus and possesses any relation to influenza is unknown.

In 1918 Park and Williams (4) made a survey of the presence of the Pfeiffer bacillus at the beginning of the influenza epidemic in New York City. Their results are given in Table 8.

TABLE 8.—*Influenza bacillus.*

INFLUENZA CASES OR ASSOCIATES.

Group.	Present.	Absent.	Present.
			Per cent.
Hospital cases	160	40	80
Marines	30	0	100
Home for children	47	1	98
AUTOPSY OF INFLUENZA CASES.			
Lungs	24	6	80
Tracheas	26	1	96
Heart's blood	3	27	10
CONTROLS.			
Nurses, contacts	4	6	40
Nurses, noncontacts	1	7	9
Measles	4	2	67
Admission ward	4	5	41
Home for girls (isolated)	2	32	6
Preventorium (not isolated)	14	25	33

These results show that a greater percentage of influenza cases harbor the influenza bacillus than do presumably healthy persons, yet they indicate the presence of this organism in normal persons. The etiologic significance of the influenza bacillus is exceedingly difficult to determine, since one set of experiments may indicate its relation to the disease while another indicates its unimportance. The very nature of influenza presents difficulties which make the etiologic significance of this organism difficult to determine. For example, we do not know the relation of influenza to the "common cold," to "grip," "catarrhal fever," or so-called "intestinal influenza," although with regard to the last it is thought that true influenza may manifest itself predominately in some cases as an intestinal form. While a vast amount of experimental work has been published purporting to establish the influenza bacillus as the actual cause of influenza, such as the experimental production by Blake and

Cecil(5) of a respiratory disease in monkeys with this organism, which they state is typical of influenza in man, we must conclude that the question remains unsettled.

Other investigators in searching for the etiologic agent of influenza have considered the possibility of a filterable virus as the cause of this disease. This is suggested by the extreme infectiousness of the disease; by the lack of uniform bacteriologic findings, particularly in the mild early cases but also in the older cases; and by the similarity of mild influenza to the common cold which has been thought by Kruse,(6) Foster,(7) and others to be caused by a filter-passing virus.

Nicolle and Lebailly(8) in 1918 reported their work on mice and guinea pigs in which they inoculated blood and secretions from cases of influenza, or uncomplicated grippe. Their results were negative. These investigators then inoculated secretions from the nose and mouth of a typical case into the conjunctiva and nasal cavities of several monkeys. Both filtered and unfiltered secretions were inoculated. At the same time two healthy human beings were inoculated. The monkeys became sick, had a temperature of 40° C., and were depressed, and diarrhœa developed within six days. The human subjects inoculated subcutaneously exhibited similar symptoms about the same time. During the same year Dujarric de la Riviere(9) injected himself intraveneously with 4 cubic centimeters of blood taken from four cases of influenza, diluted and filtered, and three days later developed a temperature of 38° C. accompanied by intense headache, chills, pains in the limbs, and weakness. After about five days he rapidly improved and remained subsequently immune to filtered sputum taken from influenza cases and sprayed into his nose and throat. About the same time Selter(10) sprayed his own throat and that of another human subject with filtered secretions taken from a patient having early influenza. Both Selter and his subject developed mild attacks of influenza. At this time Binder and Prell(11) described certain minute coccoid bodies in the tissues of influenza patients similar to those described by Noguchi in poliomyelitis. These bodies stained with iron hæmatoxylin and Giemsa's but were Gram-negative. They presented evidence that these bodies could be cultivated but were inclined to treat their etiologic significance with conservatism. Later Angerer(13) described similar bodies which he claimed to have cultivated directly from the serum of human cases of influenza.

In 1918 Broadford, Bashford, and Wilson(13) produced symptoms in guinea pigs and monkeys with filtered material taken from cases of trench fever and influenza. In anaërobic cultures they found small Gram-positive bodies which they believed to be the causative agents. In the following year Arkwright(14) reported that in his hands the cultures of Wilson proved negative when inoculated into three volunteers. During the same year Yamanouchi, Sakakami, and Iwashima(15) injected emulsions and filtrates of emulsions made of the sputa from forty-three cases of influenza, into the nose and throat of twenty-four volunteers. These authors state that six of the volunteers, who had previously had influenza, were immune and showed no symptoms following the inoculation, but the remaining eighteen developed the disease within two or three days. Furthermore, filtered blood taken from influenza patients produced the disease in healthy volunteers. These investigators were unable to produce symptoms by injecting Pfeiffer's bacillus or this organism mixed with pneumococci, streptococci, and staphylococci into the nose and throat of fourteen healthy volunteers who had never had the disease.

In 1919 Lister and Taylor(16) produced only negative results in monkeys and human beings following the injection of filtered material taken from the lungs and throats of influenza cases. Wahl, White, and Lyall(17) during the same year obtained negative results following a similar procedure. Leschke,(18) on the other hand, permitted a number of people to inhale the vapor from lung filtrates taken from typical cases of influenza and incubated for several days, and all of his subjects developed typical influenza.

An attempt to induce the disease, using filtered nasal secretions injected into healthy human subjects, met with failure in the hands of Rosenau(19) and McCoy(20) in experiments undertaken by the United States Public Health Service and the United States Navy in 1919.

From 1920 to 1923 Olitsky and Gates published a series of papers dealing with a filterable virus obtained from influenza cases. These authors used filtered and unfiltered influenza secretions. Intratracheal inoculations were made directly into the lung tissue of rabbits with unfiltered nasopharyngeal washings, the filtered washings, lung-tissue suspensions (filtered and unfiltered) from previously inoculated rabbits, and lung tissue which had been preserved in 50 per cent glycerin. The injections were made into rabbits with an intratracheal catheter, and about 3 cubic centimeters of material were administered at each injection. Within twenty-four to forty-eight hours following the inoculations the rabbits began to show symptoms. The first symptoms consisted of fever and general indisposition. A conjunctivitis and leukopenia then developed. Except in complicated cases the rabbits recovered after about three days. Clinical and pathological effects were produced in series through fifteen rabbits inoculated with ground lung tissue from a rabbit previously infected. Glycerinated material from one rabbit was passed through a series of ten rabbits, producing typical symptoms of the disease in all. The infectious agent described by Olitsky and Gates is filterable through both Berkefeld V and N candles.

When infected animals were killed during the period of illness these authors found that the lungs were enlarged and œdematous, and in some cases hæmorrhagic. Hæmorrhagic foci and alveolar exudate were found on microscopic examination.

Later Olitsky and Gates were able to cultivate from the filtered washings taken from influenza patients a minute bacilloid body which they have designated *Bacterium pneumosintes*. They have also isolated this organism from the lungs of infected rabbits. This organism is a minute Gram-negative, anaërobic bacillus-like body which is easily cultivated, but which loses its virulence rapidly under artificial cultivation. The microbe appears larger after prolonged cultivations, but cultures are still obtained from filtrates.

In summing up a discussion on the etiology of influenza in a paper on the etiology and epidemiology of influenza (1922) Zinsser(21) states:

> This leaves the entire subject in a very unsatisfactory condition. The temptation to draw definite conclusions from material of this kind is always a strong one. But to profess certainty when available evidence does not justify definite conclusions is as serious an error as to put forth inconclusive experimental work, and would serve merely to mask the truth.—The problem of influenza etiology will not, in our opinion, be solved at times when influenza epidemics are in full swing or in their secondary or tertiary waves. Solution will come from laboratories that are prepared to pounce upon the opportunity when epidemics are in their adolescence.

MacCallum(22) writing in 1926 states:

> As to influenza, the efforts of almost all the workers in the world failed to discover the etiological factor when the most gigantic material was offered but the impression became general that the bacillus of Pfeiffer

could claim nothing more than the rôle of a secondary invader and that there must be a filterable virus although no one could demonstrate it. The work of Olitsky and Gates on *Bacillus pneumosintes*, a filterable bacterium, is extremely interesting but does not convince one at once that in that organism the cause of the epidemic is found. No one has actually reproduced the disease in characteristic form in spite of many efforts and it remains an unsolved problem.

Incubation period of influenza.—The incubation period of influenza is usually considered as ranging from two to four days. In some cases it may be only a matter of a few hours and in others more prolonged.

Symptoms and clinical course of influenza.—The onset of influenza is practically always abrupt. The first symptom in many cases is headache. In some nausea is the first manifestation. The temperature begins to rise quickly and the patient feels chilly. Pains in the limbs, back, head, and burning in the eyeballs come on almost immediately and are quite marked. The temperature as a rule does not exceed 102 or 103° F. In some cases there is sore throat, catarrh, and coughing. Usually there is no rash, but slight indefinite rashes have been described as occurring in certain cases. The mortality in influenza is due chiefly to the respiratory complications since influenza itself is practically never fatal. It is believed that complications are due to secondary invaders and not to a specific etiologic agent of the disease. Early in the course of the disease there is a moderate leucocytosis and in some cases a relative lymphocytosis, but later there is a leukopenia. In some epidemics intestinal symptoms are more marked than in others. In 1889 the epidemic of influenza was characterized as a "nervous form" of the disease. During the World War the writer was stationed with the 42d Division in the Baccarat sector during what might be termed a comparatively mild epidemic of influenza. The symptoms were mostly respiratory, the disease of short duration, and many men stood by their posts throughout their illness. The relative immunity of the soldiers of the American Expeditionary Forces over that of the soldiers in the United States has been commented on above.

Animals susceptible to the virus of influenza.—Man is the natural host for this virus. Typical experimental influenza is very difficult to produce in laboratory animals, and in spite of frequent reports to the contrary, one questions whether typical influenza as it occurs in man has ever been produced in monkeys, rabbits, guinea pigs, or other laboratory animals. At present the question cannot be answered definitely.

Immunity.—There seems to be a general agreement that one attack of influenza confers a definite immunity. Vaughan (Warren) (23) in 1921 reported the results of a survey in Boston carried out during the outbreak of the disease in 1918–19. Of 2,117 families (10,000 individuals) 45 per cent had one or more persons attacked by influenza. During 1920, 14 per cent had had cases of influenza during the preceding epidemic, 58 per cent of the families has cases in either one or both epidemics, and 42 per cent of all families gave no history of influenza in either epidemic. In less than 8 per cent of 1,236 families every member of the family was attacked. This indicates that there is a natural immunity to the virus. An attack of influenza in the spring of 1918 according to Vaughan protected against a subsequent attack in the autumn. Also as has been pointed out before, the seasoned soldiers in France possessed more immunity to influenza than the unseasoned soldier at home since the American Expeditionary Forces soldier had passed through camps where influenza was prevalent and had escaped infection or had contracted a mild form of disease and had recovered. Just how long immunity to the influenza virus remains is unknown, but it is believed to last not much longer than one year.

During the World War various vaccines consisting of Pfeiffer's bacillus, pneumococci, streptococci, etc., were used for the purpose of preventing influenza and pneumonia. Jordan(24) states in reference to this question: "Protection against influenza by vaccine inoculation is evidently a broken reed on which to lean." He has published an excellent summary of its use during the last great epidemic. In general the results are conflicting and not at all promising.

Prevention.—Isolation is possible in influenza but during the war it was definitely shown that this expedient is impracticable for this disease. This is true especially during great epidemics. In the household or small hospital isolation may be practiced and may prevent further spread of the infection. Quarantine was practiced in several army units during the epidemic of 1918 as well as in civil communities, and while the spread of the disease was delayed the disease eventually spread over such barriers. Masks to be worn over the lower part of the face to prevent the spread of secretions from the respiratory tract have been employed without demonstrable effect. Complications seem to be increased in hospitalized cases, and in general this measure does not lessen the morbidity or mortality of the disease. Closing of schools has not led to a reduction in the number of cases of the disease nor has the use of nose and throat sprays and gargles. Perhaps the only measure which has any effect in preventing the spread of influenza is the avoidance of crowded places such as theaters, churches, etc., during an epidemic. Undoubtedly large gatherings of people contribute not a little to the spread of this disease. General hygienic measures are of course indicated as in the case of any other communicable disease.

BIBLIOGRAPHY

1. Olitsky and Gates, Journ. Am. Med. Assoc. 74 (1920) 1497; Journ. Exp. Med. 33 (1921) 125, 361, 373; 34 (1921) 1; 35 (1922) 1, 553.
2. Vaughan, Epidemiology and Public Health. C. V. Mosby Co., St. Louis (lit.).
3. Pfeiffer, Deutsch. med. Wchnschr. 2 (1892) 28; Zeit. f. Hyg. (1893) 13.
4. Park and Williams, Am. Journ. Pub. Health 9 (1919) 45.
   Park, Journ. Am. Med. Assoc. 73 (1919) 318.
5. Blake and Cecil, Journ. Am. Med. Assoc. 74 (1920) 170.
6. Kruse, Münch. med. Woch. 61 (1914) 1547.
7. Foster, Journ. Am. Med. Assoc. 66 (1916) 1180; Journ. Infect. Dis. 21 (1917) 451.
8. Nicolle and Lebailly, Ann. de l'Inst. Past. 33 (1919) 385.
9. Dujarric de la Riviere, Comp. rend. Acad. des Sci. 167 (1918) 406.
10. Selter, Deut. med. Woch. (1918) 932.
11. Binder and Prell, Münch. med. Woch. (1918) 1397, 1457.
12. Angerer, Münch. med. Woch. (1918) 1280.
13. Bradford, Bashford, and Wilson, Lancet 1 (1919) 169; also Qr. Journ. Med., Oxford 12 (1919) 259.
14. Arkwright, Brit. Med. Journ. 2 (1919) 233.
15. Yamanouchi, Sakakami, and Iwashima, Lancet 1 (1919) 971.
16. Lister and Taylor, Pub. South Afric. Inst. for Med. Res. (April 30, 1919) No. 12.
17. Wahl, White, and Lyall, Journ. Infect. Dis. 25 (1919) 419.
18. Leschke, Berl. klin. Woch. (1919) 11.
19. Rosenau, Journ. Am. Med. Assoc. 82 (1919) 1604.
20. McCoy, Journ. Am. Med. Assoc. 83 (1919) 401.
21. Zinsser, Medicine 1 (1922) 213 (lit).
22. MacCallum, Medicine 5 (1926) 59.
23. Vaughan, A detailed review of the epidemiology of influenza, Monograph 1. Am. Journ. Hyg., Baltimore (1921).
24. Jordan, Journ. Med. Assoc. 89 (1927) 1779.

CHAPTER X

THE RICKETTSIA DISEASES OF MAN AND ANIMALS

In 1916 da Rocha-Lima(1) designated a group of peculiar microörganisms found in lice by the term "Rickettsia" in honor of Howard Taylor Ricketts who first described organisms of this type in connection with his studies upon typhus fever. Both Ricketts and Prowazek fell victims to this malady in the course of their experimental work. Ricketts and Wilder(2) published a brief review of their work in 1910. While Ricketts was never permitted to render a complete report on his valuable work due to his untimely death, there is little doubt from the meager data available that he actually saw and described the peculiar group of microörganisms which we now designate *Rickettsia.*

In the blood of typhus-fever patients taken on the seventh to the twelfth day of the disease Ricketts and Wilder found short bacilli resembling the hæmorrhagic septicæmia group of organisms. These organisms were stained by Giemsa's method. Their length was estimated as about 2 micromillimeters and their diameter at about one-third of this figure. On close examination a faintly stained bar was seen to extend across the middle. Organisms were occasionally seen end to end. There were also observed in these preparations stained granules that were thought by Ricketts to be degeneration or involution forms of the organism. Some of the larger granules or "small rods" stained deep purple, while the smaller ones were colored blue. The bacillary bodies were not motile but were observed to vibrate more or less rapidly. These authors also examined the dejecta of lice for the presence of these organisms. They state—

> Streptococci, staphylococci, and oval bacilli occurring in clusters, and certain solid bacilli, are encountered irregularly and indifferently in the feces and intestinal contents of both "normal" and "infected" (lice which were fed on typhus fever patients) lice. Polar staining organisms have been found occasionally in the feces and intestinal contents of normal lice, whereas they are present almost constantly, and often in large numbers, in similar material from "infected" individuals.

Gavino and Gerard(3) during the same year also described bacilliform bodies and bipolar bodies free in the blood plasma of typhus cases. Hegler and Prowazek(4) in 1913 described from several cases of typhus fever certain paired granules in neutrophilic leucocytes that they believed to be microörganisms. Also in one instance these authors found a small coccuslike double organism in a smear from a louse fed upon typhus cases. The first definite work associating rickettsia and typhus was that of da Rocha Lima in 1916. This author found these organisms in sections of lice which were fed on typhus cases but was unable to demonstrate their presence in normal lice. These bodies were present within the epithelial cells of the louse's stomach, and there was some evidence that the cells were damaged by the presence of the organism. Da Rocha Lima described these bodies as organisms consisting of two substances, one staining faintly with Giemsa's and the other taking a deep stain at the poles. Under the dark field these bodies resembled paired granules. Da Rocha Lima states that the single granules measure 0.3 by 0.4 micron, while the double granules or rods measure 0.3 by 0.9 micron. He designated these organisms *Rickettsia prowazeki* in honor of Prowazek.

These results were soon confirmed by Nöller,(5) Töpfer and Schüssler,(6) and by Otto and Dietrich.(7) The latter also described threadlike forms of rickettsiæ in recently infected lice. In 1916 Töpfer(6) described rickettsiæ in lice in association with trench fever and this work was soon confirmed by Munk and da Rocha Lima,(8) although these authors denied its relationship to the disease and denied its pathogenic properties. These authors suggested the name *Rickettsia pediculi* for this organism. Brumpt(9) in 1918 and Strong(10) in 1919 opposed the idea that these rickettsiæ were pathogenic. Both fed lice containing *Rickettsia pediculi* upon healthy persons without producing any ill effects. By this time it was known that some rickettsiæ are pathogenic while others are not, and it had been shown that some rickettsiæ are intracellular while others are extracellular. In 1909 Ricketts(2) described "bodies" which he found in the blood of human and experimental cases of Rocky Mountain spotted fever and also in the tissues and eggs of infected ticks. Wolbach(11) in 1919 definitely associated these organisms with rickettsiæ. In 1923 Sellards(12) cultivated from a monkey infected with tsutsugamushi fever an organism which he believes resembles rickettsia. In 1925 Cowdry(13) described *Rickettsia ruminantium,* of heartwater disease of sheep, goats, and cattle, while in 1928 Sellards and Siler(14) presented evidence to show that dengue fever may also be caused by an organism of this type.

That certain rickettsiæ are pathogenic while others are nonpathogenic is now a well-established fact. Rickettsiæ, both pathogenic and nonpathogenic, have been found in a variety of insects. The diseases for which etiological agents of this type have been established are as follows: Typhus fever, *Rickettsia prowazeki;* Rocky Mountain spotted fever, *Dermacentroxenus rickettsi;* heartwater, or veldt sickness, *Rickettsia ruminantium;* and possibly in trench fever, *Rickettsia pediculi;* tsutsugamushi fever, *Rickettsia nipponica,* and dengue fever (unnamed). The latter have not as yet been definitely established.

In addition to the pathogenic rickettsiæ mentioned a number of organisms that also appear to be of this type have been described and apparently are unrelated to disease processes. To Table 9, from Wolbach, Todd, and Palfrey, we have added a few rickettsiæ that have been described since 1920.

It will be noted in Table 9 that a surprisingly large number of rickettsiæ have been described since the early work of Ricketts in 1909. According to Hertig and Wolbach, over forty rickettsia-like organisms have been reported. Authorities differ upon the nature of these "bodies," and even though several of them have been the subject of much study we are unable to state just what is their exact nature. Woodcock believes that these "bodies" represent granules which result from disintegration or lysis of red blood corpuscles or other cells. Others believe that rickettsiæ represent actual microörganisms. It is quite possible that certain granules resulting from the disintegration of red blood cells and other cells may resemble closely the so-called rickettsiæ, and the true rickettsiæ may in reality be microbic in nature. Cowdry(15) defines rickettsiæ as including Gram-negative, intracellular, bacterium-like organisms. Whether or not they are bacteria cannot be definitely asserted. There is, however, little evidence to favor the theory of their being Protozoa. In connection with the classification of the rickettsiæ with the filterable viruses Cowdry(16) states that "Very obviously the rickettsiæ, which have been sometimes included in this category, belong elsewhere." However, even though as a group the rickettsiæ present characteristics that are common to each other and permit their specific group classification, there still remains the possibility that some of these organisms may be filterable. The Trench Fever Commission of the American Red Cross stated, as one of their conclusions from the study of this disease, that "The organism causing

TABLE 9.—*Rickettsiæ and their insect hosts.*[a]

- Insecta.
  - Mallophaga.
    - *Melophagus ovinus* (sheep "louse" or "tick").
      - *Rickettsia melophagi* Nöller (1917).
  - Corrodentia.
    - *Psocus* sp.? (dust louse).
      - Unnamed rickettsia Sikora (1918).
  - Hemiptera.
    - *Pediculus humanus* (human louse).
      - *Rickettsia prowazeki* Hegler and Prowazek (1914); da Rocha Lima (1916).
      - *Rickettsia (rocha-lima?)* Weigl (1920).
      - *Rickettsia pediculi* Munk and da Rocha Lima (1917).
      - *Rickettsia quintana* Munk and da Rocha Lima (1917); Schmincke (1917).
      - *Rickettsia wolhynica* Toepfer (1916).
    - *Cimex (Acanthia) lectularius* (bedbug).
      - *Rickettsia lectularius* Bacot (1921).
  - Diptera.
    - *Culex pipiens* (mosquito, Europe).
      - Unnamed rickettsia Nöller (1920).
    - *Aëdes ægypti* (mosquito, Philippines, etc.).
      - Unnamed rickettsia Sellards and Siler (1928).
  - Siphonaptera.
    - *Ctenocephalus felis* (cat flea).
      - *Rickettsia ctenocephali* Sikora (1918).
    - *Ctenopsylla musculi* (mouse flea).
      - Unnamed rickettsia Sikora (1918).
- Arachnida: Acarina.
  - *Dermacentor venustus* (wood tick, United States).
    - *Dermacentroxenus rickettsi* Ricketts (1909); Wolbach (1919).
  - *Leptus (Trombidium) akamushi* (harvest mite, Japan).
    - Unverified quotation by Sikora (1920).
    - *Rickettsia nipponica* Sellards (1923); Nagayo (1924).
  - *Dermanyssus* sp.? (bird mite, Europe).
    - Unnamed Nöller (1920).
  - *Amblyomma hebraeum* (bont tick).
    - *Rickettsia ruminantium* Cowdry (1925).

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[a] Hindle, Parasitology 13 No. 2 (1921) 152, has reported, without description, the existence of rickettsiæ in lice of the horse, *Trichodectes pilosus*, and of the goat, *Linognathus stenopsis*. To these new rickettsiæ he has given the names *Rickettsia trichodectæ* and *Rickettsia linognathi*, respectively.

the disease is a resistant, filtrable virus." The members of this commission were unable to produce trench fever with filtrates prepared from infected blood but were successful in three of five instances with filtrates prepared from infected urine and fæces. They state: "The virus causing trench fever, therefore, is evidently filterable at one stage at least of its life cycle, and in this respect it resembles the viruses of typhus, and of phlebotomus, and dengue fevers, to all of which diseases trench fever bears some analogy." Furthermore, these authors found that the virus of trench fever is not to be found within the epithelial cells of the gut of the louse but is present in the lumen and consequently in the dejecta. In typhus fever the virus is found within the epithelial cells of the gut of the louse and can be regarded as an intracellular organism.

In general we may say that the rickettsiæ represent a comparatively new group of organisms and should be classified as a special group of microörganisms, at least for the time being. They should be defined as Gram-negative, bacterium-like organisms, some of which are intracellular and some of which are extracellular, some of which may be filterable, and most of which are apparently nonfilterable, and some of which are pathogenic and related to specific disease processes, while others are harmless.

BIBLIOGRAPHY

1. DA ROCHA LIMA, Arch. f. Schiffs- und Tropen-Hyg. Leipsig **20** (1916) 17; Berl. klin. Woch. **53** (1916) 567; Münch. med. Woch. **64** (1917) 33; Deut. med. Woch. **45** (1919) 732; Ergeb. d. allg. Path. und. path. Anat., Lubarsch and Ostertag, Wiesbaden (1919) 159.
2. RICKETTS, Journ. Am. Med. Assoc. **52** (1909) 379.
   RICKETTS and WILDER, Journ. Am. Med. Assoc. **54** (1910) 1304, 1373.
3. GAVINO and GERARD, Mexico City, Publicaciones del Instit. Bact. Nacional, No. 2 (May 20, 1910); No. 3 (June 10, 1910).
4. HEGLER and PROWAZEK, Berl. klin. Woch. **1** (1913) 2035.
5. NÖLLER, Berl. klin. Woch. **53** (1916) 778; Arch. f. Schiffs- und Tropen-Hyg. **21** 53.
6. TÖPPER and SCHÜSSLER, Deut. med. Woch. **42** (1916) 1157.
   TÖPFER, Berl. klin. Woch. **53** (1916) 323; Deut. med. Woch. **42**: 1251.
7. OTTO and DIETRICH, Deut. med. Woch. **43** (1917) 577; Centralbl. f. Bakt. etc., I, Abt., Orig. Jena **82** (1918) 383.
8. MUNK and DA ROCHA LIMA, Münch. med. Woch., No. 44 (1917) 1422.
9. BRUMT, Bull. Soc. Path. Exotique **11** (1918) 249.
10. STRONG, Report of Commission, Medical Research Committee, American Red Cross. Oxford Press (1918); The Significance of Rickettsia in Relation to Disease. Contributions to Medical and Biological Research, dedicated to Sir Wm. Osler, N. Y., Paul Hoeber **2** (1919) 1205.

11. WOLBACH, Journ. Med. Res. 41 (1919) 1.
12. SELLARDS, Am. Journ. Trop. Med. 3 (1923) 529.
13. COWDRY, Journ. Exp. Med. 42 (1925) 231, 253; 44 (1926) 803.
14. SELLARDS and SILER, Am. Journ. Trop. Med. 8 (1928) 299.
15. COWDRY, Journ. Exp. Med. 37 (1923) 431.
16. COWDRY, Journ. Bact. 13 (1927) 20.

TYPHUS FEVER

Definition.—Typhus fever is an acute self-limited, specific, infectious disease caused by *Rickettsia prowazeki,* and is transmitted to man through the agency of lice.

History and distribution.—Typhus fever is thought to have existed in the time of Hippocrates. It is a matter of record that this disease was confused with typhoid fever until early in the nineteenth century. Before that time typhus fever was rampant in several parts of the world and large epidemics of typhoid fever were practically unknown. During the nineteenth century typhus fever began to decrease while great epidemics of typhoid fever became common. With the beginning of the World War typhus fever again appeared in epidemic proportions in Russia, Serbia, Poland, Germany, Bulgaria, and parts of Mexico. Typhus fever according to its history has always been associated with poverty, famine, vermin infestation, and pestilence of war. This was true during the Thirty Years' War and again during the last World War. It has been estimated that of one hundred seventy-five thousand British seamen in service during the Revolutionary War over eighteen thousand died of this disease. In Mexico typhus fever has long been endemic, at times assuming epidemic proportions. It is still present in that country. It has been estimated that nearly one hundred fifty thousand people died of this disease in Serbia during 1915. In the United States the disease has never become epidemic. There was a small outbreak of the disease in Philadelphia in 1883 and one in New York in 1891. Since 1900 there have been a few cases reported in different years, the greatest number being thirty-five reported in 1916. Most of these cases have proved to be among recently arrived immigrants from Europe.

At the present time typhus fever exists in Europe, Asia, Africa, and America. It is commonest in Russia, the Balkans, Poland, Ireland, Galicia, Mexico, and Spain. In America it was formerly known as Brill's disease.

The virus.—The rickettsiæ are small, bacterium-like bodies, and in the opinion of some investigators they may be related to the protozoans. These organisms are transmitted to man by various insects. They stain best with Giemsa's or Romanowski's stain and are found in clusters or pairs within the intestinal cells of the louse in typhus fever. Similar organisms are found in the intestinal cells of *Dermacentor andersoni,* a tick which transmits Rocky Mountain spotted fever to man. In trench fever, another disease which may be due to a similar organism, the virus is apparently extracellular.

The rickettsia causing typhus fever is found in the circulating blood of cases of this disease but does not pass through the pores of a Berkefeld filter. The organisms do not grow on artificial culture media, but are cultivable in tissue culture. They are killed by heating to 55° C. for fifteen minutes but are not destroyed by freezing. Da Rocha Lima(1) described this organism as the cause of typhus fever in 1919 and named it after Ricketts and Prowazek. This agent is now thought to be the true etiologic cause of the disease.

In 1920 the Report of the Typhus Fever Research Commission of the League of Red Cross Societies to Poland was published.(2) This commission made extensive investigations upon the etiology and transmission of the disease. It will be recalled that Brumpt and Strong were able to demonstrate rickettsiæ in lice from presumably disease-free persons, and were unable to infect persons with such rickettsia-infected lice. These control experiments were based wholly upon the examination of smear preparations. Munk and da Rocha Lima pointed out that there were two types of rickettsia in lice, one extracellular, which multiplies in the lumen of the gut, and the other intracellular, associated with typhus which multiplies within the epithelial cells. The latter was named *Rickettsia prowazeki* while the former was designated *Rickettsia pediculi.* The Typhus Fever Research Commission states—

> In no control work has it been shown that intracellular rickettsia occur in lice from a certainly typhus free population, although both da Rocha-Lima and Toepfer state that very rarely *Rickettsia pediculi* may invade the cells. We, in view of the heavy typhus infestation of the countries in which this work was done, and our failure to find intracellular rickettsia in the controlled British stock lice fed upon Mr. Bacot during his illness with trench fever, regard these observations as open to strong doubt. Twenty-six lice of the British stock fed upon Mr. Bacot during his illness were studied in section. Seventeen contained extracellular rickettsia. Nine were negative. In none did we find intracellular rickettsia. On the other hand, since the association of rickettsia with trench fever has been given etiological significance by Toepfer (1916), Munk and

da Rocha-Lima (1917), Arkwright and Bacot (1919), (3) Byam (1919), (4) and since the rickettsia observed by these authors are of the extracellular proliferating type and indistinguishable from *Rickettsia pediculi* of da Rocha-Lima, the question of deciding the specificity of rickettsia for trench fever is a most difficult one. The query is: are *Rickettsia quintana* (*wolhynica*) and *Rickettsia pediculi* identical? If so, is this rickettsia the cause of trench fever?

The commission believes that there is strong presumptive evidence for this conclusion.

According to the Typhus Fever Commission *Rickettsia prowazeki* ranges in size from 0.25 by 0.45 micron to 0.3 by 1.1 microns. These measurements correspond closely to those which have been given by da Rocha Lima for these organisms. The commission states:

> In all smears, in addition to the small forms described above we have noted the presence of small numbers of slightly coccoid paired bodies, somewhat lanceolate in shape and devoid of bluish staining intermediate substance. These forms can be recognized in sections of lice and are the forms most easily demonstrable in human tissues.

The commission also found that the delicate filamentous forms appeared only in lice fed upon typhus patients and never in control lice. These forms were definitely accepted as *Rikettsia prowazeki.*

Sikora(5) in 1920 reported the presence of rickettsia in the salivary gland, but these findings were not confirmed by the Typhus Fever Commission. The commission inoculated a series of twelve guinea pigs with fæces heavily infected with *Rickettsia prowazeki,* and concluded from the temperature reactions that in five instances the animals contracted typhus fever. Infection in guinea pigs was later confirmed by histologic examination. Da Rocha Lima (1919) had been unable to infect guinea pigs with louse fæces, although Nicolle, Blanc, and Conseil(6) in 1914 had reported successful infections in these animals. Strong in 1918 showed that the virus of trench fever is not transmitted by infective lice to their ova although a number of observers had described *Rickettsia pediculi* as occurring in the ova. The Typhus Fever Commission found no rickettsiæ in smear preparations of ova, larvæ, and nymphs from eight boxes which contained lice infected with *Rickettsia prowazeki.*

In 1912 Nicolle and Conor(7) reported that monkeys, *Macacus rhesus,* were resistant to typhus fever. This observation was confirmed by the Typhus Fever Commission, although they were able to produce a temperature reaction with the same material used for the *Macacus rhesus* monkeys in one *Cercopithicus* (*sabra ?*). Two strains of typhus were maintained in guinea pigs for a period of over a year, the virus being transmitted from guinea pig to guinea pig by intraperitoneal injection of heart's blood. The virulence of the virus was unaffected by repeated passage. The commission made numerous attempts to cultivate the virus of typhus without success. In human tissues the virus was demonstrated in the blood vessels in the skin, brain, kidneys, muscles, and testes. In general the commission concludes that the criteria for the diagnosis of *Rickettsia prowazeki* are as follows: The size; morphology; their staining reactions which must correspond with those of *Rickettsia prowazeki* in sections of lice; and their presence in vascular endothelial cells in situ in relation to the lesions of typhus.

Later Wolbach and Schlesinger(8) demonstrated that the virus of typhus survives in tissue-plasma cultures for a length of time corresponding to the life of the endothelial cells multiplying in the cultures. The microörganism can be found in the endothelial cells in large numbers. Also brain-tissue cultures from typhus guinea pigs have proved successful. Plasma from guinea-pig blood is collected in paraffin-lined tubes, centrifuged, and chilled. The tissue for cultivation is cut in blocks 0.5 to 1 cubic millimeter and is kept immersed in Ringer's solution, then transfered to a sterile cover slip, and covered with sterile plasma. In this way the virus may be kept alive from four to six weeks.

Incubation period.—The incubation period in typhus fever varies between four and fourteen days, the average being twelve days. In guinea pigs the incubation period ranges from seven to ten days.

Symptoms.—The period of onset lasts about two days. This period is characterized by nausea, headache, vertigo, etc. In some cases a general convulsion may occur and pass into delirium. The temperature usually rises suddenly on the third day to 103 or 104 ° F. The face becomes congested and the eyes suffused. The tongue becomes coated and the breath foul. The facial expression becomes dull and stuporous. Epistaxis and vomiting may occur. The rash appears on the fifth day and is generally pleomorphic. It has been described by the term "mulberry rash." It first appears upon the abdomen and inner aspects of the arm and later spreads over the chest, back, and trunk. In some cases it may involve the soles of the feet and hands as well as the face. It may persist for ten days and usually fades slowly.

Complications are common, especially in cases that are neglected. A terminal bronchopneumonia is common. Abortion, parotitis, and bedsores are frequent complications.

In most cases the lethargy is pronounced. This is spoken of as the "typhus state." Cerebration is slow, hands and tongue are tremulous, muscle twitchings may come on (cortical irritation), and the patient may sink into a low muttering delirium. Secretion of urine may be diminished, suppressed, or there may be incontinence. Constipation rather than diarrhœa is the rule. There is a moderate leucocytosis ranging from 12,000 to 15,000. The breath has been described as having the odor of gun washings.

Animals susceptible to the virus of typhus.—Man is the natural host for the virus of typhus, the virus being transmitted from man to man through the agency of the louse *Pediculus humanus* var. *corporis* and *capitis.* The virus is pathogenic for chimpanzees, lower monkeys, and the guinea pig.

Immunity.—One attack of typhus fever confers a definite immunity. Animals experimentally infected that recover possess a well-defined immunity against subsequent infection. Many guinea pigs are said to be naturally immune. This is also true of some species of monkeys.

In 1915 Weil and Felix isolated certain strains of the proteus group of bacteria from the stools and urine of typhus-fever patients which they designated X2 and X19. The serum of typhus-fever patients possesses the property of agglutinating these organisms, but it has been demonstrated beyond doubt that *B. proteus* is not the cause of the disease. The reaction, however, is of diagnostic significance, especially since it appears usually before the sixth day of the disease. This reaction has been designated the "Weil-Felix" reaction. The serum when tested between the eighth and twenty-first days may have an agglutinating power of 1 : 2,500.

Pathology.—The anatomical changes in typhus fever consist chiefly of lesions in the blood vessels. There is a perivascular infiltration of cells and a disintegration of the endothelium and proliferation causing thrombi and hæmorrhages. Lesions of the blood vessels of the skin result in the characteristic macular and hæmorrhagic eruption. Similar lesions are also found in the brain from which result the symptoms related to the central nervous system. Similar lesions are also found in the blood vessels of the kidneys, muscles, and testes.

Prevention and control.—Preventive and control measures should be directed toward the eradication of the body louse. Isolation and quarantine are of course indicated during epidemics. The problem has a large social aspect which involves the education of the ignorant along hygienic lines and the betterment of the economic status of the poorer classes who are most affected by this disease. Proper clothing and food are important considerations in epidemics of this disease, and all articles of wearing apparel of affected persons should be effectively sterilized as well as rooms which they occupy. Asheshov has introduced a serum treatment in southern Russia which is said to mitigate the severity of the disease although it does not shorten its duration. The serum employed by Asheshov is obtained from convalescent petients. No satisfactory vaccine or serum is available for protection.

BIBLIOGRAPHY

1. Da Rocha Lima, Deut. med. Woch. 45 (1919) 732.
2. Wolbach, Todd, and Palfrey, Report of the Typhus Fever Research Commission of the League of Red Cross Societies to Poland, Harvard University Press, Cambridge, Mass. (1922) (lit.).
3. Arkwright and Bacot, Journ. Hyg. 18 (1919–21) 76; Trans. Soc. of Trop. Med. and Hyg. 12: 61.
4. Byam, Trench Fever. Oxford University Press, London (1919).
   Byam and Lloyd, Proc. Royal Soc. of Med. 13 (1919) 1–20.
5. Sikora, Archiv f. Schiffs- und Tropen-Hyg. 24 (1920) 347.
6. Nicolle, Blanc, and Conseil, Arch. de l'Inst. Past. de Tunis 9 (1914) 184.
7. Nicolle and Conor, Bull. Soc. Path. Exotique 5 (1912) 460.
8. Wolbach and Schlesinger, Journ. Med. Res. 44 (1923–24) 231.

TRENCH FEVER

Definition.—Trench fever is a specific infectious disease which is caused by a filterable virus transmitted by the louse *Pediculus humanus* var. *corporis.* It is characterized by recurrent pyrexia, headache, vertigo, sweating, and polyuria. There is a slight leucocytosis at the height of the fever.

History and distribution.—Trench fever first appeared in the British Expeditionary Force in 1915. In the beginning this disease was diagnosed P. U. O. (pyrexia of unknown origin). Later it became known as "trench fever." During the remaining years of the World War the disease spread to the French, Italian, German, and Austrian armies. Some epidemiologists have attempted to indentify this disease with quintan fever, described by Hippocrates, Galen, and others, but there is little information upon which to base such a conclusion. The report of the Trench Fever Commission of the American Red Cross Committee(1) in 1918 asserts that "it may be stated that no other infectious disease during the past two years has occasioned so much sickness among the troops in France." Grieveson(2) reported that trench fever formed 40 per cent of his hospital evacuation, and at one time 60 per cent of all cases of sickness. Further, this author stated that the morbidity rate of trench fever can be equalled only by such plagues as typhus, typhoid, and malaria. Muir(3) reported that within eight months there were three hundred fifty cases of trench fever sent to three field ambulances each month.

The virus of trench fever.—From the researches of the Trench Fever Commission of the American Red Cross Research Committee there seems to be little doubt that the causative agent of this disease is a filterable virus. The conclusions reached by this commission are quoted below:

1. That trench fever is a specific, infectious disease; that it is not a modified form of typhoid or paratyphoid fever, and is not related, from an etiological standpoint, to these diseases.
2. That the organism causing the disease is a resistant filterable virus.
3. That the virus causing trench fever is present particularly in the plasma of the blood of trench fever cases, and that such plasma will produce the disease on inoculation into healthy individuals.
4. That the disease is transmitted naturally by the louse Pediculus humanus, Linn., var. corporis, and that this is the important and common means of transmission. That that louse may transmit the disease by its bite alone, the usually manner of infection, or the disease may be produced artificially by scarifying the skin and rubbing in a small amount of the infected louse excrement.
5. That a man may be entirely free from lice at the time he develops trench fever, the louse that infected him having left him sometime previously as its host, and that the louse need only remain upon the individual for a short period of time in order to infect him.
6. That the virus of trench fever is also sometimes present in the urine of trench fever cases, and occasionally in the sputum, and that the disease may be produced in man by the introduction of the virus in the urine or sputum through the scarified or otherwise abraded skin.
7. That since the urine and sometimes the sputum of trench fever patients are infective, these should be sterilized in order to avoid the possibility of accidental infection from them.
8. That in order to prevent trench fever or limit its spread, and thus save man-power for the armies, greater effort must be made to keep soldiers in general from infestation with lice.

The British Investigation Committee(4) found that lice remain infective for at least twenty-three days after feeding on the infected patients. Probable infection is brought about by rubbing and scratching the skin upon which is deposited the louse and its excreta.

The Trench Fever Commission inoculated thirty-four men either with blood or some constituent element of the blood, taken from trench-fever cases in the febril stages of the disease. The disease was induced in twenty-three of these cases. McNee(5) and his colleagues reported that the virus of trench fever was intracorpuscular and not free in the plasma or serum. The Trench Fever Commission, however, showed that the virus is present in the fluid part of the blood, and is not contained within the blood corpuscles themselves. No evidence that the virus is filterable was obtained by the commission from five experiments performed with filtered plasma, and one with filtered serum. However, a typical attack of the disease was produced with filtrates prepared from the sediments of infected urines. Also the disease was produced in two out of three instances with filtered material prepared from the excrement of infected lice. The commission states: "Therefore, these three positive experiments demonstrate that at least one stage of development of the virus of trench fever is filterable and ultramicroscopic."

The commission also demonstrated that the virus of trench fever resists a temperature of 60° C. moist heat for thirty minutes but is destroyed at 70° C. moist heat when exposed for the same length of time.

As has been true in many other diseases of obscure etiology a large variety of etiological agents have been suggested as the cause of trench fever. McNee, Brunt, and Renshaw suggested the intracorpuscular nature of the causative agent of this disease but did not favor the theory that it is ultramicroscopic. Houston and McCloy(6) suggested the enterococcus as the causative factor. These authors succeeded in isolating this organism from the blood of three cases of trench fever, from the urine of three, and from the sputum of one. Sundell and Nankivell,(7) however, were unable to isolate this organism from the blood of cases having this disease. Dimond(8) described a protozoan, a hæmogregarine, resembling *Hæmogregarina gracilis* found by Wenyon in a lizard host. This organism is said to have been found in twelve cases from the venous blood and in material obtained by splenic and liver puncture. These results have not been confirmed. Henry (9) described a flagellate similar to the organism described by Dimond. Pappenheimer, Vermilye, and Muller (10) described a small, circular, slightly oval, bluish purple body lying among the blood cells and free in the plasma, although these authors later decided that these bodies were not related to the disease in an etiological way. Sundell and Nankivell (11) described a spirochæte which they found in the urine of cases of trench fever, an observation that was confirmed by Patterson (12) and by Stoddard. (13)

His (17) described diplococci and short rods free in the plasma and in the red cells in the blood of Wolhynian fever. Similar bodies were found also in guinea pigs that were inoculated with blood from cases of trench fever. These bodies stained at the poles. Such organisms were also described by Zollenkopf, (18) Brasch, (19) Jungmann and Kuczynski, (20) and others. Jungmann and Kuczynski believed that these bodies belong to the Chlamydozoa and are similar to *Rickettsia prowazeki* of typhus fever. The same bodies were found in the intestines of lice fed upon their cases. Da Rocha Lima (21) and Brumpt, (22) however, have found similar bodies in the intestines of normal lice fed upon healthy individuals, and such lice when fed upon other healthy individuals produce no disease. The thought persisted, however, that trench fever is caused by *Rickettsia pediculi.* Investigations by Toepfer, (23) Munk and da Rocha Lima, (24) Arkwright and Bacot, (25) and Byam (26) indicate that trench fever is caused by a rickettsia. Toepfer found rickettsia-like organisms in association with Wolhynian fever and described diplobacillary and diplococcoid bodies in the blood of trench-fever patients. In another paper he reported the presence of these bodies in lice fed upon trench-fever cases. Munk and da Rocha Lima also found rickettsiæ in lice from trench-fever cases, but these authors were unable to prove their relation to the disease since these bodies were found in lice fed on normal people. Similar results have been reported by Arkwright and Bacot, and the question of deciding the specificity of rickettsia for trench fever is difficult. Byam states:

> "In fifty-three experimental inoculations of volunteers at the New End Hospital, the lice or excreta of lice, used as virus, were examined microscopically. In every case the lice had previously been fed on a trench fever case. Twenty-seven specimens showed rickettsia bodies and caused trench fever. Ten specimens did not show rickettsia and did not cause trench fever. One specimen showed rickettsia, but did not cause trench fever. Two specimens did not show rickettsia bodies, but caused trench fever." Four were doubtful. Nine specimens showed rickettsia bodies,

but did not cause trench fever because the virus had been destroyed by heat or disinfectant.

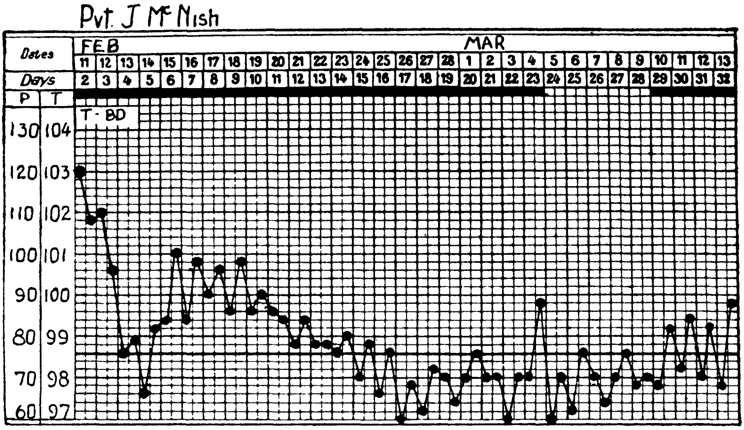
This author concludes, "The constant presence, after a suitable lapse of time, of rickettsia bodies in lice which have fed on a trench fever patient has been confirmed. The absence of rickettsia bodies from lice bred in captivity, and fed only on healthy men, has been shown by our work."

The Trench Fever Commission of the American Red Cross Research Committee concludes that no visible organism is demonstrable, and that the virus is filterable and should be classified as a resistant filterable agent. They point out that the difficulties encountered in filtration of the trench-fever virus are common to many other filterable viruses and this alone should not mitigate against the filterable virus etiology of this disease. Also the resistance of the virus to heat is characteristic of other viruses that are accepted as filterable agents.

While some believe that the cause of trench fever is a rickettsia decision must at present be held in abeyance. *Rickettsia pediculi* is found in the intestinal lumen of the louse and not in the epithelial cells as in the case of the rickettsiæ of typhus fever and Rocky Mountain spotted fever. From a survey of the literature bearing upon this subject we are inclined to the view that *Rickettsia pediculi* may be nonpathogenic and as such is not related in an etiological way to trench fever. For the present, at least, we must think of trench fever as a virus disease and leave future investigations to decide the exact nature of the causative agent.

Incubation period.—The incubation period in trench fever is extremely variable. In artificially induced infections the incubation period has varied from five to thirty days. In general it ranges from fourteen to thirty days.

Symptoms.—There are prodromal symptoms consisting of headache, pain in the limbs, fever, etc., during the incubation period. The disease comes on suddenly and is characterized by headache, dizziness, pains in the legs, back, and behind the eyeballs, injection of the conjunctivæ, and a sharp rise in temperature to 103 or 104° F. The fever is of a relapsing character in most cases. In the great majority of cases, 70 to 80 per cent, an erythematous rash develops over the chest, back, and abdomen. In some cases the rash is papular. The rash usually disappears within twenty-four hours. Usually the erythematous spots appear early in the course of the disease. The urine may show albumin. The leucocytic count is not constant.



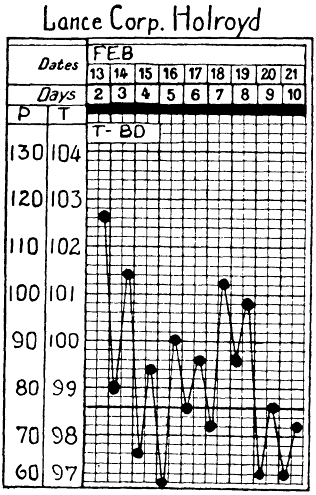
Pvt. J. McNish.

Admitted Feb. 11, 1918. Onset sudden, Feb. 12th, at 2 p.m. In the morning he felt a little dizzy; after dinner a little weak, headache, backache, and pain in upper part of thighs. There was slight diarrhoea during the day, but none to-day. Last night sleepless because of the pains. There was dryness of the throat with some slight trouble in swallowing. This morning he vomited; temperature 103° F. Pains continue; prostration seems to be more marked. Physical examination: Tonsils enlarged, not red; no exudate; slight tenderness left side of neck, just under angle of the jaw. Spleen palpable, heart and lungs normal. Slight supra-orbital tenderness; marked tenderness in the lumbar regions and in the upper outer side of thighs. Feb. 13. He is much better to-day and is hungry. Feb. 15. Not so comfortable as yesterday. The pain in the lower lumbar regions continues. Feb. 17. There is moderate tenderness and slight rigidity in lower right abdomen. Feb. 18. Had a very good night, free from pain to-day; the tenderness in the back continues. Feb. 21. Has only a slight pain in the back. Mar. 26. Has had no further symptoms. Convalescence has been uneventful except for some increase in heart action, and slight fever. Summary: Short-course type of trench fever, followed by two slight febrile relapses without symptoms when patient got up. On Feb. 12th, the third day of disease, his blood was transferred to No. 22 (Schaeffer), who developed long-course trench fever after an incubation period of five days. Lice were fed on McNish, then transferred to No. 72 (Cody). After remaining on Cody two days they were transferred to No. 14 (Lowell). Both of these subjects developed trench fever after a period of twenty-six and thirty days respectively.

FIG. 5. Case of trench fever. (After the Trench Fever Report of Commission Medical Research Committee, American Red Cross.)

There may be a moderate leucocytosis or no rise at all. The fever is variable. It may persist for a week, then fall, or it may be intermittent in type. Trench-fever cases are prone to have relapses. Some cases may have six or seven relapses with periods of several weeks intervening between relapses. In nearly every case there is an enlargement of the spleen.

Usually the patient recovers within five to six weeks, but the disease may be prolonged over long periods of time. A certain percentage of patients pass into chronic ill health that is manifested by nervous symptoms, mental depression, disordered ac-



Lance-Corp. Holroyd, age 34.

Admitted Feb. 13, 1918. Perfectly well yesterday morning until 9 a.m., when there was a sudden onset, with pain in head, back, and legs. He vomited several times, had a troublesome cough, and was kept awake most of last night with pain and cough. There was very marked prostration from the time of onset. Symptoms continued; he reported sick, and was sent immediately to the hospital. Physical examination: Marked prostration, flushed face, coated tongue, throat normal, heart over-active, pulse bounding. Lungs: no dullness; few sibilant and sonorous sounds, especially over left posteriorly. Abdomen held somewhat rigid, but relaxes on breathing. Spleen palpable. Tenderness: supra-orbital, lumbar, thighs, knees, and in shins. Feb. 14. Had a restless night, with headache, backache, and pains in the legs. There is some pain over the heart, less coughing, appetite better, lungs clear. Tenderness: supra-orbital, lumbar, over splenic area, over upper side of thighs, very marked in the shins and knees. Feb. 15. Is much better; has very little pain. Herpes has appeared on lips. Feb. 18. Had a definite clinical relapse yesterday afternoon, but this morning is feeling better. Mar. 1. Has had no further relapses; very little pain. His convalescence has been steady. Summary: Typical short-course type of trench fever. On Feb. 14, the third day of disease, his blood was transferred to No. 35 (Walker), who developed long-course type of trench fever with an incubation period of five days.

FIG. 6. Case of trench fever. (After the Trench Fever Report of Commission Medical Research Committee, American Red Cross.)

tion of the heart, etc. Trench fever is never fatal, and complete recovery is the rule.

Animals susceptible to the virus of trench fever.—Man alone is affected by trench fever. Experimental work in animals with various viruses supposed to be the causative agent of this disease has been reported, but at present such work cannot be accepted as well authenticated or related to the true virus of trench fever.

Immunity.—There seems little doubt that some immunity is produced by one attack of trench fever. The exact degree of immunity, however, that is conferred is unknown. The fact that relapses are so prone to occur in this disease indicates that the degree of immunity produced is not very great.

Pathology.—There has been a great paucity of material with which to study the anatomical changes produced in trench fever. It is known, however, that the lesions are largely vascular and similar to those in typhus fever and Rocky Mountain spotted fever.

Control measures.—Prevention and control of trench fever is brought about by delousing, and the disinfection of urine, sputum, and other secretions of the patient that are infective.

BIBLIOGRAPHY

1. Trench Fever Report of Commission of Medical Research Committee of the American Red Cross. Oxford University Press (1918).
2. GRIEVESON, Lancet 2 (1917) 84.
3. MUIR, Brit. Med. Journ. (Nov. 11, 1916) 641.
4. BRITISH INVESTIGATION COMMITTEE, Brit. Med. Journ. (Jan. 19, 1918) 91; (Mar. 9, 1918) 296.
5. MCNEE, BRUNT, and RENSHAW, Brit. Med. Journ. 1 (1916) 225.
6. HOUSTON and MCCLOY, Lancet (Oct. 7, 1916) 632.
7. SUNDELL and NANKIVELL, Lancet (Mar. 16, 1918) 401.
8. DIMOND, Lancet (Sept. 8, 1917) 382.
9. HENRY, Brit. Med. Journ. (Dec. 1, 1917) 739.
10. VERMILYE and MULLER, Brit. Med. Journ. (Oct. 13, 1917) 474.
11. SUNDELL and NANKIVELL, Lancet (1917) 416, 672.
12. PATTERSON, Brit. Med. Journ. (Sept. 29, 1917) 418.
13. STODDARD, Brit. Med. Journ. (1917) 416.
14. MCCREA and DICKSON, Lancet (May 26. 1917) 796.
15. CHANDLER, Lancet 1 (1916) 461.
16. DYKE, Lancet 2 (1916) 767.
17. HIS, Berl. klin. Woch. (July, 1916) 738.
18. ZOLLENKOPF, Deutsche med. Woch. (Aug. 24, 1916) 1034.
19. BRASCH (1916). Reviewed in Bull. Internat. d'Hyg. Publ. 8: 1219.
20. JUNGMANN and KUCZYNSKI, Deutsche med. Woch. (Mar. 22, 1917) 359; Zeitschr. f. klin. Med., Berlin 85 (1917) 251.
21. DA ROCHA LIMA, Deutsche med. Woch. 42 (1916) 1353.
22. BRUMPT, Bull. de la Société de Pathologie Exotique 11 (1918) 249.
23. TOEPFER, Berl. klin. Woch. 53 (1916) 323; Deut. med. Woch. 42: 1251; Münch. med. Woch. 63: 1495.
24. MUNK and DA ROCHA LIMA, Münch. med. Woch. No. 44, (1917 1422–26.
25. ARKWRIGHT and BACOT, Journ. Hyg. 18 (1919–21) 76.
    ARKWRIGHT, BACOT, and DUNCAN, Trans. Soc. of Trop. Med. and Hyg. 12 (1919–22) 61.
26. BYAM, Trench Fever. Oxford University Press, London (1919).
    BYAM and LLOYD, Proc. Roy. Soc. Med. 13 (1919) 1.

ROCKY MOUNTAIN SPOTTED FEVER

BLACK FEVER; BLUE DISEASE

Definition.—Rocky Mountain spotted fever is a specific infectious disease supervening on the bite of a certain tick, *Dermacentor andersoni,* that is focalized on the western slope of the Bitter Root Valley in Montana. The disease is characterized, as is typhus fever, by a macular eruption which becomes petechial. Increased irritability and hyperæsthesia of the skin are common, the spleen is enlarged, the urine may contain albumin and casts, and catarrh of the respiratory tract is present throughout the course of the affection.

History and distribution.—Rocky Mountain fever is said to have existed along the Snake River in Idaho since 1873. In 1889 Maxey(1) published what is thought to be the first descrpition of the disease. It was not until 1902 that any serious effort was made to study this affection. At that time Wilson and Chowning,(2) at the request of the State Board of Health of Montana, began an investigation of the disease in the Bitter Root Valley. As a result of their work it was learned that the disease occurs chiefly in the spring of the year, and these authors discovered that a tick was instrumental in spreading the infection. Wilson and Chowning believed that the disease was caused by a hæmatozoan related to *Piroplasma* and suggested the name *"Piroplasmosis hominis"* by which to designate it.

Rocky Mountain fever is most prevalent and fatal in the Bitter Root Valley, but the infection has spread over several states in the Rocky Mountain region, including Colorado, Idaho, Utah, Oregon, Nebraska, Montana, and Wyoming, and has been found in California, South Dakota, Washington, and British Columbia. In Idaho the disease is of a mild character, while in Montana its mortality has been as high as 90 per cent. About five hundred cases are reported each year.

In 1906 Ricketts(3) confirmed the finding of Wilson and Chowning that the virus of Rocky Mountain fever is carried by a tick and further demonstrated that the particular tick involved is *Dermacentor andersoni.* Other ticks, *Dermacentor modestus* and *D. marginatus,* are also said to be capable of transmitting the infection. The disease may be transmitted from man to monkeys and to guinea pigs.

The virus of Rocky Mountain fever.—The virus of Rocky Mountain fever is transmitted to man through the bite of the tick *Dermacentor andersoni.* The infection may be transmitted by the larva, the nymph, and both adult female and male ticks, Only the adult tick attacks man, but the disease is transmitted to animals such as the mountain goat, sheep, brown bear, coyote, badger, and wild cat, by the larvæ and nymphs. The virus is also transmitted by heredity through the ticks to their larvæ.

Wolbach(4) first described the virus of Rocky Mountain fever, although Ricketts had previously described certain "bodies" present in the blood stream of human cases as well as in the tissues and eggs of infected ticks. Wolbach described small bodies which stained with Giemsa's in the endothelial cells of the blood vessels. These he also found in the testes of man, in experimentally infected guinea pigs, and in the bodies, salivary glands, and eggs of infected ticks. Lanceolate diplococcal organisms were found in the circulating blood and in the endothelial cells. These bodies contain a chromatin-staining substance. Also blue-staining rod-shaped forms were noted. This author has designated these organisms *Dermacentroxenus rickettsi.* Later Wolbach(5) in collaboration with Schlesinger succeed in cultivating these organisms in tissue-plasma medium (see Typhus Fever).

The virus of Rocky Mountain spotted fever is readily inoculated into man and can be passed to monkeys and guinea pigs, both of which exhibit a characteristic symptom; that is, scrotal inflammation and swelling. Under natural conditions comparatively few ticks are infected. Ricketts found only one in two hundred ninety-six ticks. Ricketts explains the difference between the case mortality rate in Montana (case fatality of 90 per cent) and in Idaho (case fatality of about 5 per cent) on the basis of there being a particular species of tick capable of transmitting the infection in each area. In Montana *D. venustus* is the vector, while in Idaho *D. maturatus* carries the infection. (*Dermacentor venustus* and *D. andersoni* refer to the same species.)

The virus of Rocky Mountain spotted fever is not filterable. It is less bacterium-like than any of the rickettsiæ. Cross-immunity experiments with the virus of typhus fever, according to Wolbach, Todd, and Palfrey,(6) show that guinea pigs that have recovered from typhus fever do not react as do normal guinea pigs to the virus of Rocky Mountain spotted fever. Typhus-fever-immune guinea pigs develop spotted fever, but they all show lengthened incubation periods and lower temperatures. The mortality is also reduced about 50 per cent.

The characteristic form of the virus is a short rod, which occurs in pairs and clusters in the lesions of the blood vessels, testicle, skin, and subcutaneous tissues. It invades all the tissues of the tick, and a rickettsia-like organism has been cultivated from the tick by Noguchi.(7) In man the virus is found chiefly in the smaller peripheral blood vessels and causes a proliferating endarteritis.

Symptoms.—The disease is ushered in by general malaise followed by chills. The temperature rises from the second to the fifth day from 103 to 105 or 107° F. Delirium and semi-unconsciousness develop. In favorable cases the temperature begins to fall by lysis by the end of the second week. The eruption appears about the fourth to the seventh day. The eruption is macular in character and later becomes petechial with a tendency to become confluent. Slight jaundice of the skin and scleræ is also present. Gangrene of the fingers, toes, lobes of the ears, and skin of the elbows may occur in some cases. The spleen is enlarged and tender, the liver showing only slight enlargement. Urine is scanty, highly colored, and may contain albumin and casts. Nausea and vomiting usually occur in the second week and persist throughout in unfavorable cases. Respiration and pulse are rapid. There is usually no increase in leucocytes, but an increased number of mononuclear cells. Gangrene of the tonsils, scrotum, and prepuce is commoner in the mild form of the disease than in the highly fatal type. No explanation has been offered for this. Constipation rather than diarrhœa is the rule.

The disease somewhat resembles typhoid fever but is easily differentiated from typhoid by its sudden onset and a negative Widal reaction.

Animals susceptible to the virus of Rocky Mountain fever.—In addition to man the following animals have been found to be hosts for the tick *D. andersoni, D. maturatus,* etc.: Monkeys, guinea pigs, rabbits, ground squirrels, woodchucks, goats, sheep, the brown bear, the coyote, badgers, and wild cats. The virus of spotted fever has been transmitted to monkeys, guinea pigs, rabbits, ground squirrels, and woodchucks.

Immunity.—Spotted fever attacks either sex, and at any age. One attack of the disease confers a high degree of immunity. Laboratory animals are completely immune following an attack of the disease. No case of a second attack in man has been reported. Blood serum from recovered cases possesses protective properties for guinea pigs. Also the serum from convalescent animals is protective. Noguchi(8) has been able to immunize animals with a mixture of convalescent serum and virus. Spencer and Parker(9) found that emulsions of infected ticks to which is added 0.5 per cent phenol, will protect not only guinea pigs, rabbits, and monkeys against the virus of spotted fever, but there is good evidence that human beings can be immunized as well by this method.

Pathology.—The anatomical changes in spotted fever consist of a hypostatic congestion of the lungs, enlarged spleen and lymph glands, subserous petechiæ, fatty degeneration of the hepatic cells, congestion of the renal cortex, and hæmorrhages into the genitalia. Gangrene of the scrotum and prepuce is frequently noted. There is a proliferation of the endothelium of the arteries and veins of the skin and subcutaneous tissue, and a perivascular mononuclear infiltration. The rickettsia bodies are found within the endothelial cells.

Prevention and control.—Effort to prevent and control spotted fever should logically be directed toward the eradication of the ticks that serve as carriers of the virus. Dipping and spraying of domestic animals have been recommended. These together with clearing of the land by burning, and cultivation of the tillable soil should do much in lowering the incidence of this disease. The protective vaccine of Spencer and Parker, if as efficacious as their early reports indicate, should be employed extensively for prophylaxis.

BIBLIOGRAPHY

1. Maxey, Portland Med. Sentinel (Oct., 1889).
2. Wilson and Chowning, Journ. Infect. Dis. 1 (1904) 31.
3. Ricketts, Trans. Chicago Path. Soc. 7 (1906) 73.
4. Wolbach, Journ. Med. Res. 34 (1916) 121; 41, 1.
5. Wolbach and Schlesinger, Journ. Med. Res. 44 (1923) 231.
6. Wolbach, Todd, and Palfrey, The Etiology and Pathology of Typhus. Harvard University Press, Cambridge (1922).
7. Noguchi, Journ. Exp. Med. 43 (1926) 515.
8. Noguchi, Journ. Exp. Med. 38 (1923) 605.
9. Spencer and Parker, U. S. Pub. Health Rep. 39 (1924) 3027; 40 (1925) 2159.

HEARTWATER: VELDT SICKNESS

Definition.—Heartwater, or veldt sickness, is an acute infection characterized by hydropericardium and an acute gastroenteritis, presumably a rickettsia disease transmitted from affected animals to healthy animals by the tick *Amblyomma hebraeum.*

History.—Heartwater, according to Hutcheon, (1) first appeared in South Africa in 1860. This was about the time the bont tick, *Amblyomma hebraeum,* appeared in the same locality. The disease has been studied experimentally by Hutcheon, (2) Edington, (3) Cowdry, (4) and others. Edington succeeded in transmitting heartwater to susceptible cattle by inoculating blood containing the virus; Lounsbury (5) transmitted the disease to sheep and goats.

Distribution.—Heartwater has been limited to South Africa, but in 1918 the disease was reported in Angola and the Belgian Congo by Van Saceghem. (6) The disease has not been reported in any other locality.

Incubation period of heartwater.—The incubation period in heartwater is approximately fourteen days in the natural infection by the tick, but is usually somewhat less when the disease is induced artificially by inoculated blood. It may vary from five to fifteen days.

Symptoms of the disease.—The first sign of the disease is fever. This reaction is characteristic, the temperature rising abruptly to 105° or more and remaining at this level for as long as a week after which it may suddenly drop to subnormal before death. Theiler (7) believes that the symptoms can be explained only by the fact that the virus is present in the blood stream. Nervous symptoms such as muscular twitching, squinting, salivation, tetanic seizures, and galloping movements after the animal has fallen are said to occur occasionally. (Cowdry.) According to Theiler the mortality is over 50 per cent.

Animals susceptible to the virus of heartwater.—The disease appears to be limited to sheep, goats, and cattle. No other species has been experimentally infected.

The virus of heartwater.—That the virus of heartwater is present in the circulating blood has been adequately demonstrated. According to Theiler (8) the virus is not filterable through a Berkefeld or Chamberland filter. Cowdry, at the suggestion of Theiler, recently made a cytological study of the tissues of experimentally infected animals (goats) in order to test the hypothesis that the disease is due to a rickettsia. According to Cowdry's protocols the heartwater virus used in his experiments transmitted the disease to goats with great constancy. The incubation period in the artificially induced disease was about ten days. Sheep and cattle were also used in some of the experiments. Cowdry was unable to demonstrate any organisms in the fresh blood from infected animals, though it was known that the blood was infective. However, in fixed tissues, at first from the spleen and later from other organs, peculiar Gram-negative cocci were demonstrated. These microorganisms were found in the endothelial cells of the capillaries of the renal glomeruli, and in the superficial gray matter of the cerebral cortex. Microörganisms were also found in the lymph glands, corpus luteum, cerebellar cortex, suprarenals, mid-brain, medulla oblongata, ovaries, corpus striatum, salivary glands, pancreas, and heart muscle. The organisms were never demonstrated in either the liver or the lungs. After death the organisms remained in the tissues for at least six hours.

Cowdry states that the most characteristic lesion in the disease is a marked swelling of the endothelial cells. In these cells the organisms may be found in great numbers. While isolated organisms were never observed, the clumps were always found to be surrounded by a halo of cytoplasm staining very lightly or not at all. In some cases the endothelial enlargements were found entirely blocking the lumina of the capillaries.

These organisms are described by Cowdry as "uniform coccus-shaped bodies, 0.2 to 0.5 micra in diameter, as measured after fixation in Zenker's fluid or Rebaud's fluid and coloration by Giemsa's stain, or by any basic anilline dye." These bodies are clearly differentiated from the granules in granular leucocytes, from mitochondria, from the granules of mast cells, and from the products of the phagocytosis of hæmoglobin. Cowdry believes the organism to be a typical endothelial parasite and has designated it *Rickettsia ruminantium* since it is the first microorganisms resembling rickettsiæ found associated with a disease of ruminants. In a second paper Cowdry has studied *Rickettsia ruminantium* in the tick and found that—

> when larvae, which had taken no food since hatching, were allowed to feed upon cases of heartwater, they acquired Rickettsiae which appeared to be identical with those in the tissues of animals suffering from heartwater, whereas control larvae hatched from eggs deposited by the same female and fed on normal animals remained free of Rickettsiae. After larvae presumably infective had molted, the resultant nymphae containing Rickettsiae in their alimentary tracts, when fed upon susceptible animals produced in them typical attacks of heartwater, which the control nymphae, devoid of Rickettsiae, failed to do.

The tissues of the infected animals were found by this author to contain typical rickettsiæ, thus presenting the final argument for the etiology of this disease.

Immunity in heartwater.—One attack of the disease confers immunity. Hyperimmunized cattle, sheep, and goats produce an effective serum for their respective species, but practical immunization has not been established. The supposition of Edington that the disease is identical with horse sickness is considered by Theiler as incorrect, as horse sickness can be transmitted artificially only to horses. (Hutyra and Marek.)

Control measures for heartwater.—Control measures for heartwater consist in prevention by the eradication of the tick that transmits the rickettsia.

BIBLIOGRAPHY

1. HUTCHEON, Agr. Journ. Cape of Good Hope 17 (1900) 410.
2. HUTCHEON, Agr. Journ. Cape of Good Hope 19 (1901) 302; 20 (1902) 633; 22 (1903) 438.
3. EDINGTON, Agr. Journ. Cape of Good Hope 17 (1904); Comp. Path. and Therap. 17 (1904) 141.
4. COWDRY, Journ. Exp. Med. 42 (1925) 231–252; 42 (1925) 253–274; 44 (1926) 803–814.
5. LOUNSBURY, (1899) Agr. Journ. Cape of Good Hope 17 (1900) 682; (1902) 20, 29; 21, 22, 165, 221, 315; Rep. Gov. Entomol. (1903) 15; Agr. Journ. Cape of Good Hope (1904) 14.
6. VAN SACEGHEM, Bull. Soc. Path. Exot. 11 (1918) 423.
7. THEILER, Ann. Rep. Gov. Vet. Bact. (1903–04) 114; Vet. Journ. 9 (1904) 300; Rept. Transvaal Dept. Agr. 1903–04 (1905) 190; Ann. Rep. Dir. Agr. 1904–05 (1906) 121; Ann. Rep. Gov. Vet. Bact. 1905–06 (1907) 67; (1909) 33.

TSUTSUGAMUSHI: JAPANESE FLOOD FEVER

Definition.—Tsutsugamushi disease is a specific, infectious disease distributed along the course of the large rivers in Japan, and characterized by fever, chills, tenderness and swelling of the lymph glands, and a generalized cutaneous eruption. The disease is transmitted by a mite known locally as akamushi. This disease has been thought to be due to a filterable virus, and there is some evidence that it is caused by a rickettsia.

History.—"Tsutsugamushi" signifies "dangerous bug" and according to Kawamura (1) it was spoken of in a Chinese medical book written in the Sei era, in the sixth century, as a "sand-louse." In "Honso Komoku" Lishiting in the sixteenth century wrote—

> The sand-louse is found along river banks during the summer. It is tiny, not easily discerned as the tip of a hair, and is red. If one walk along the river bank, or through weeds, or bathe in the water in the morning or evening, after a rain, the mites will attach themselves to one's skin and bore into it. It becomes reddened, and if one rub the

> reddened area with the hand, there is a sharp pain, like the pricking of a needle. Within three days all the joints become painful, chills and fever appear, and pox form where the skin was red.

The disease was undoubtedly present in China in former times but is no longer found there.

The disease was first described scientifically in 1878 by Bälz and Kawakami (2) who studied the disease in Japan and named it "river fever" or "flood fever." In 1908 the disease assumed epidemic proportions in the eastern part of Formosa, and was definitely identified by Hatori (3) in 1914 as tsutsugamushi disease. In 1920 Kawamura demonstrated that the disease in Formosa is identical with the disease as it occurs in Japan. Similar diseases have been described by Schuffner (4) in Sumatra; by Ashburn and Craig (5) in the Philippines; by Dowden (6) in the Malay Peninsula; and by Smith in Mossman, North Queensland. Whether these reports represent true instances of tsutsugamushi is not definitely known. Such names as "Deli pseudotyphoid" and "atypical tsutsugamushi" have been applied to these conditions which simulate true tsutsugamushi.

Distribution.—According to Kawamura, tsutsugamushi is found in the flood basins of the large rivers of Japan. This author states—

> In Niigata prefecture there are three localities along the Shinano and Akano rivers; in Akita prefecture one along the Omono river; and in Yamagata prefecture one along the Mogami river. While the disease occurs in the flood districts of certain rivers, it does not in those of others. It is found chiefly in Niigata prefecture. In Formosa it occurs not only along the rivers, but also in forest and reclaimed land.

The virus of tsutsugamushi.—Kitasato (7) in 1893 described a small body in the red corpuscles of a patient suffering from tsutsugamushi that he thought was a plasmodium. This could not be confirmed by other investigators. In 1904 Tanaka, (8) who had previously thought the disease to be caused by a protozoan, claimed to have identified a proteus at the site of the insect bites. Later this author stated that he believed the disease was caused by the toxin of the mite akamushi. Ogata and Ishiwara (9) in 1905 claimed to have found an amœboid protozoan in both patients and cadavers, and in the following year stated that their organism was a sporozoan for which they were able to produce an immune serum. In 1910 these authors described the life cycle of this parasite and named it "*Gregarina tsutsugamushi.*" Later Ogata stated that he believed the parasite was not a protozoan but a schizomycete. Hayashi(10) in 1908 described rod-shaped, spheroidal, and ring-shaped bodies in the blood cells and tissue around the site of the akamushi bite, and in subsequent publications states that these bodies are protozoans similar to *Theileria parva,* the cause of coast fever in cattle which is prevalent in East Africa. He has named these bodies *Theileria tsutsugamushi.*

Kitashima and Miyajima(11) have described small spherical bodies in the akamushi that parasitize field rats. These bodies are also found in the pupæ and larvæ. In 1917 these authors found certain granular bodies in the akamushi, and on the surface of agar cultures that they designate akamushi bodies. According to Kawamura these akamushi bodies are undoubtedly yeast cells.

In 1917 Nagayo, Miyakawa, Imbamura, and Tamiya(12) cultivated a bacterium from an infected monkey which when injected into susceptible monkeys produced tsutsugamushi disease. Agglutination tests with this bacterium however were negative. Ishiwara and Ogata, Jr.(13) demonstrated small, round organisms in the lymph nodes, spleen, heart, and in monkeys having the disease, which they were able to cultivate. Later, in 1924, Nagayo found short rod-shaped bodies resembling rickettsiæ that stained with azure II but did not stain by Giemsa's method. Sellards(14) in 1923 infected a monkey with tsutsugamushi material and cultivated an organism which resembles *Rickettsia nipponica.* Kawamura points out that—

> It is, however, strange that the inoculation of these cultures into his (Sellards) animals brings about hemorrhagic conditions never observed in our series of several thousand experiments. Furthermore the results he (Sellards) obtained in the monkey were insufficiently striking to prove that he had isolated the true causative agent of tsutsugamushi disease.

The virus of tsutsugamushi disease is present in the lymph nodes and in the blood stream of infected monkeys. The virus tends to disappear during convalescence. One one-hundredth cubic centimeter of infected blood is sufficient to infect a susceptible monkey, and in certain cases 0.001 cubic centimeter may be sufficient to induce the disease. The virus passes through a Berkefeld filter, and blood so filtered is capable of inducing the infection. The virus apparently will not pass through a porcelain filter. Serum after clotting of infected blood does produce the disease. Centrifugation of infected blood plasma for one-half hour at 2,000 to 3,000 revolutions per minute does not completely remove the infectious agent. Kawamura has been unable to wash red cells from infected blood free of the infectious agent, and he believes that the infectious agent is closely attached to the formed elements of the blood. From his experiments he concludes that the infectious agent is not related to blood platelets, and severer symptoms were obtained with washed leucocytes than with washed red blood cells. He states that "when the number of leucocytes is so adjusted that it corresponds to the number of leucocytes in a measured amount of blood, the degree of illness produced in either case will be about the same." Tsutsugamushi disease appears to be similar to typhus fever in some respects, but in typhus fever the infectious agent is apparently found in the blood corpuscles while this is not true in flood fever.

The causative agent in flood fever is not present in the vesicles produced upon patients with blistering plasters; it is not present in spinal fluid or urine from cases of the disease or in the fœtal blood from infected mothers. The virus of flood fever is destroyed by heating for ten minutes at 55° C.; it remains viable at 25° C. for one week; it is viable from three to five days at 37° C.; drying for twelve hours destroys its infectivity; it is destroyed by pure glycerol, 1 per cent mercuric chloride, 1 per cent phenol, and 2 per cent potassium hydroxide. Bile and ascitic fluid also apparently affect the virus, especially after a short incubation in these mixtures. Distilled-water mixtures with infected blood also attenuate or destroy the virus after twelve hours incubation at 25° C.

It is said that the virus of tsutsugamushi will remain viable for a time in the blood of infected guinea pigs, but these animals exhibit no symptoms of the disease

Monkeys are easily infected with tsutsugamushi virus and have a definite incubation period ranging from six to thirteen days. The infected animal presents a typical fever curve and a marked leukopenia. Furthermore, the monkey develops an immunity to the disease. Artificially produced acites in infected monkeys contain the virus which when introduced into other susceptible monkeys induces the disease.

Incubation period.—The incubation period of tsutsugamushi fever ranges from four to seven days. In some cases the disease develops within three days following infection, while in others the incubation period has extended to twelve days. In monkeys the incubation period ranges between six and thirteen days.

Symptoms.—In the early stages of the disease cases of tsutsugamushi exhibit general malaise, headache, a feeling of oppression, anorexia, insomnia, and, according to Kawamura, photopsia. Some cases may show dizziness, pain in the joints, and epistaxis. There are fever, chills, and constipation. Tenderness and swelling of the lymph glands are characteristic of the disease. Skin necrosis and crusts are noted. These occur at the site of the invasion and are thought to be due to the secretion of the akamushi. The rash reaches its full development in from three to five days and consists of both macules and papules. It is generalized over the entire body and is neither itching nor painful. The eruption lasts from five to nine days and fades gradually, or rapidly in some cases, leaving no trace, while in others it may leave brownish spots or crusts. The mortality ranges between 10 and 60 per cent.

Animals susceptible to tsutsugamushi virus.—Man appears to be the natural host for this virus. Monkeys may be infected experimentally.

Immunity.—One attack of tsutsugamushi confers some immunity, but the disease may be contracted more than once. The second attack is usually mild though in some cases the second attack may be as severe as the first or even more marked. Monkeys become immune following artificial infection. Complement-fixation studies have been unsatisfactory, and Wassermann tests performed on cases of this disease in which syphilis could be ruled out have been negative. The Weil-Felix reaction in tsutsugamushi is negative.

Pathology.—There are no specific visceral changes in tsutsugamushi disease. There are general signs of septicæmia which somewhat resemble those of typhoid. The spleen and liver are swollen, and there is cloudy swelling in the parenchymatous organs and cellular necrosis. There is also an increase in the reticular-endothelial elements. In some cases hæmorrhagic changes are found in the lungs. There is no marked change in the pancreas, thyroid, or reproductive glands.

Control measures.—Since the disease is transmitted to man by the bite of the akamushi the eradication of this mite is essential for the control of the disease. The bodies of men working in infested districts should be sprayed with 1:100 "Desin" or neosol three times a day. (Kawamura.) Protective suits that cover the entire body except the eyes have also been devised in an effort to protect men in infested areas.

BIBLIOGRAPHY

1. KAWAMURA, The Medical Bulletin, College of Medicine, University of Cincinnati, Nos. 1 and 2, **4** (1926) (lit.).
2. BÄLZ and KAWAKAMI, Virchow's Arch. f. path. Anat. **78** (1879) 373. BÄLZ, Virchow's Arch. f. path. Anat. **78** (1879) 528.
3. HATORI, Taiwan Igak. Zas. (1915–20) Nos. 147, 150, 170, 182, 209, and 153.
4. SCHÜFFNER, Nederl. Tijdschr. v. Geneesk. **2** (1913) 1141; Münch. med. Wchnschr. **3** (1913) 158.
5. ASHBURN and CRAIG, Boston Med. and Surg. Journ. **158** (1908) 749; Philip. Journ. Sci. § B **3** (1908) 1.
6. DOWDEN, Ind. Med. Gaz. **1** (1915) 208.
7. KITASATO, Tokyo Igak. Zas. **7** (1893) No. 20; **9** (1895) Nos. 3 and 4 (Jap.).
8. TANAKA, Centralbl. f. Bakt. u. s. w. 1ste. Abt. Orig. **26** (1899) 432; **42** (1906) 16, 104, 235, 329.
9. OGATA and ISHIWARA, Deutsch. med. Wchnschr. (1909) No. 33; Mitt. d. med. Fak. d. Kaiserl., Univ. z. Tokyo **7** (1907) 205; **9** (1910) 175; **10** (1911) 155.
10. HAYASHI, Hokuetsu Igak. Zas. (1908) No. 165 (Jap.); Beitr. z. Hyg. Bakt. u. Infektionskr. (1910) 46.
11. KITASHIMA and MIYAJIMA, Saikingaku Zas. (1910) No. 166 (Jap.); Kitasato Arch. Exp. Med. **2** (1918) 91.
12. NAGAYO, MIYAKAWA, IMAMURA, and TAMIYA, Tokyo Igak. Zas. (1915) No. 29 (Jap.); Journ. Exp. Med. **25** (1917) 255; Am. Journ. Hyg. **1** (1921) 569.
13. ISHIWARA and OGATA, Jr., Centralbl. f. Bakt. u. s. w. 1ste. Abt. Orig. **90** (1923) 164.
14. SELLARDS, Am. Journ. Trop. Med. **3** (1923) 529.

ILLUSTRATIONS

PLATE 24

FIG. 1. *Rickettsia prowazeki*, from gut contents of louse. (After Typhus Research Commission of the League of Red Cross Societies to Poland.)

2. *Rickettsia prowazeki*, showing swollen and vacuolated epithelial cells packed with granular rickettsiæ. (After Typhus Research Commission of the League of Red Cross Societies to Poland.)

PLATE 25

Section showing swollen and vacuolated epithelial cells of midgut filled with *Rickettsia prowazeki.* The lumen of the gut infected with larger, more deeply staining rickettsiæ. These, like the organisms which cover the cuticular border of the gut cells, are *Rickettsia pediculi.* (After Typhus Research Commission of the League of Red Cross Societies to Poland.)

PLATE 26

Section of skin (low power). From a case of Mexican typhus showing the proliferative lesions, "typhus nodules," and a cross section of an artery with a thrombus. (After Typhus Research Commission of the League of Red Cross Societies to Poland.)

PLATE 27

FIG. 1. Trench fever. Dressing of volunteer after removal of the cell itself, without cleaning. Note the soiling of the skin with louse excrement, especially at the distal border of the cell and about the ulcerated area. (After the Trench Fever Report of Commission, Medical Research Committee, American Red Cross.)

2. Trench fever. Feeding by the box method; higher up is seen the circular impression left by the rim of the box and the macular eruption within it caused by the recent bites of lice; encrusted papules resulting from previous feedings are also present. (After the Trench Fever Report of Commission, Medical Research Committee, American Red Cross.)

PLATE 28

FIG. 1. Heartwater. Kidney showing large blood vessel with engorged endothelial cells in which the microörganisms, *Rickettsia ruminantium,* are arranged in characteristic clumps. (After Cowdry.)

2. Spleen showing swollen endothelial cell containing mass of microorganisms, *Rickettsia ruminantium,* embedded in chromophobic cytoplasm. (After Cowdry.)

PLATE 29

FIG. 1. Tsutsugamushi fever. Eruption in a woman of 22, taken six days after onset. (After Kawamura.)

2. Tsutsugamushi fever. Sketch of eruption on the arm of a man of 30. (After Kawamura.)

PLATE 30

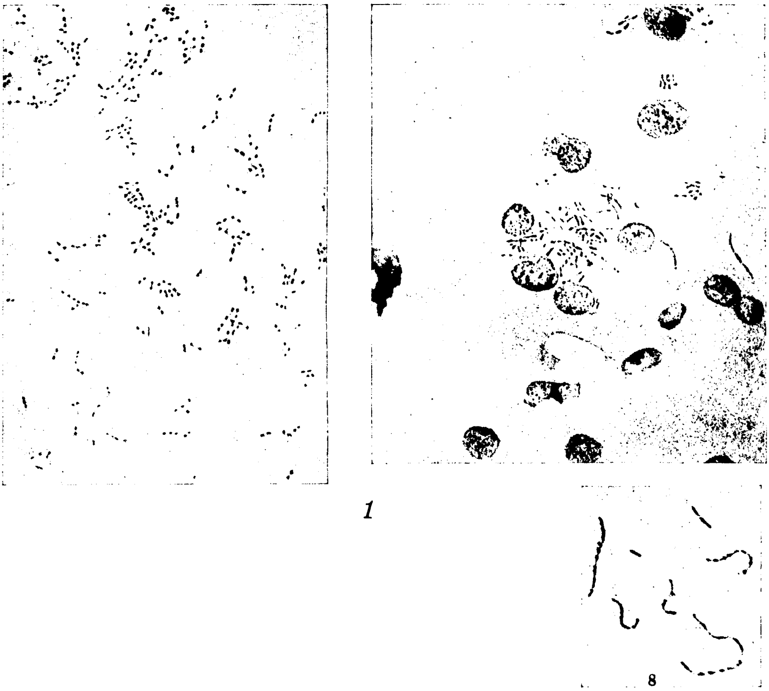
FIG. 1. Tsutsugamushi fever (human case). Bite lesion with the akamushi in situ, sucking organ evident. (After Kawamura.)

2. Tsutsugamushi fever. Microörganisms cultivated from animals infected with tsutsugamushi virus. (After Sellards.)

PLATE 31

FIG. 1. Tsutsugamushi fever (human case). There is so much cellular infiltration near the bite wound that this may be considered a bite of an infected akamushi, or a primary lesion of the disease. (Photomicrograph by Kawamura.)

2. Tsutsugamushi fever. Men, protected by vermin-proof suits, catching field voles. (After Kawamura.)



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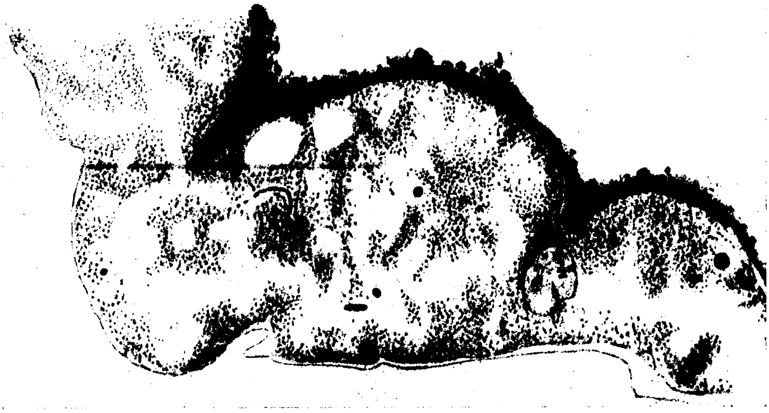

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Fig. 1. From gut contents of louse. 2. Showing swollen and vacuolated epithelial cells packed with granular rickettsiæ.

PLATE 24. RICKETTSIA PROWAZEKI.

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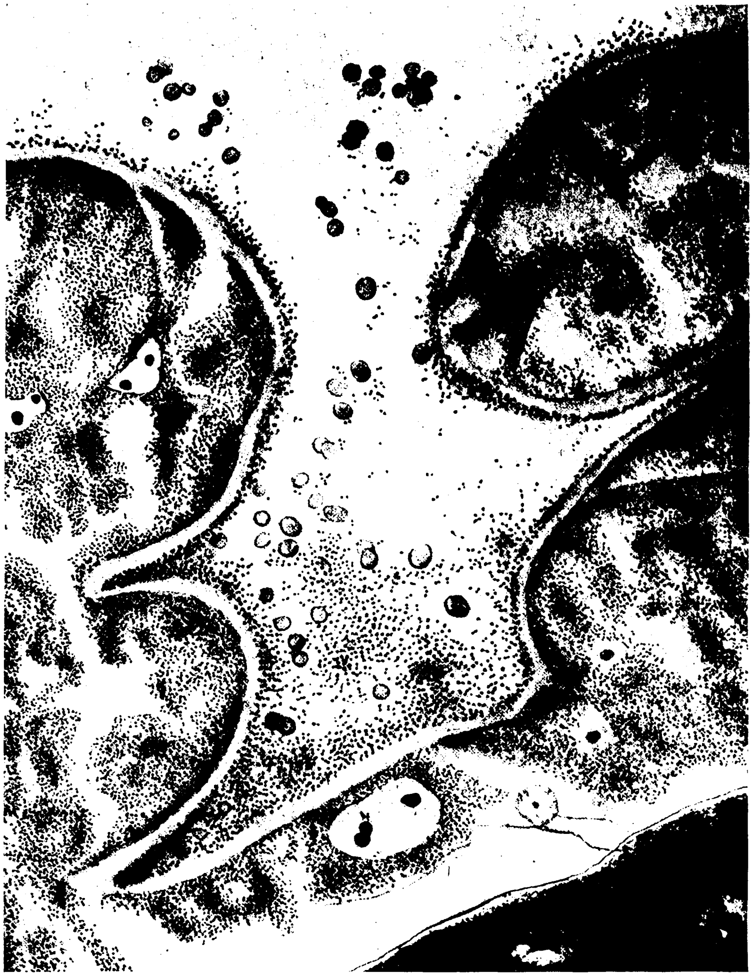

PLATE 25. SECTION SHOWING SWOLLEN AND VACUOLATED EPITHELIAL CELLS OF MIDGUT FILLED WITH RICKETTSIA PROWAZEKI.

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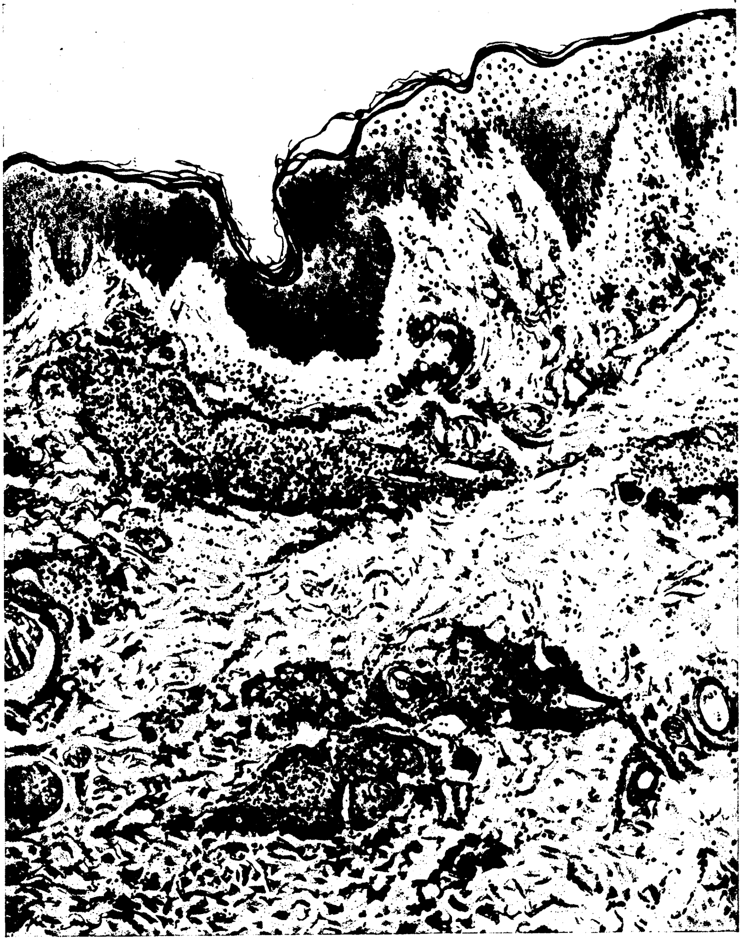

PLATE 26. SECTION OF SKIN (LOW POWER). FROM A CASE OF MEXICAN TYPHUS SHOWING THE PROLIFERATIVE LESIONS, "TYPHUS NODULES," AND A CROSS SECTION OF AN ARTERY WITH A THROMBUS.

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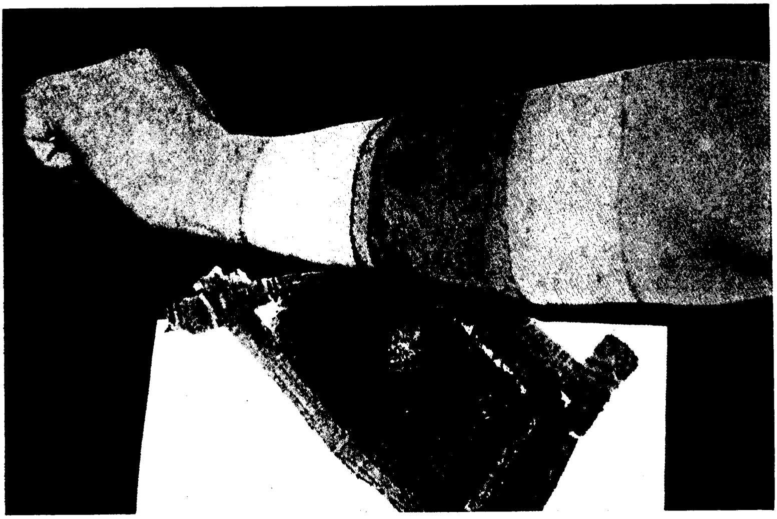

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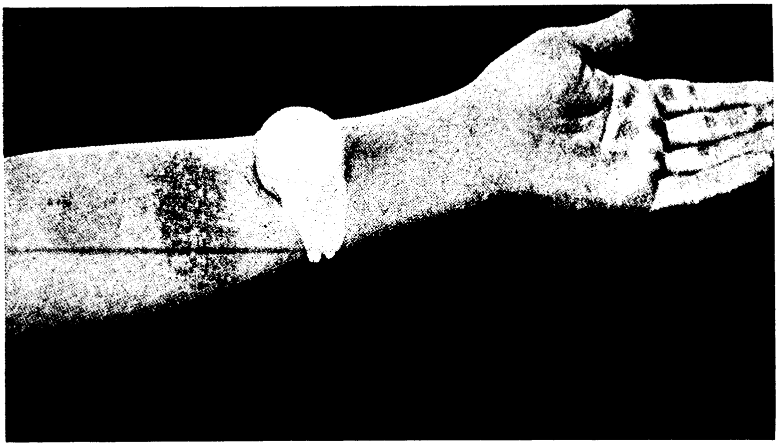

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Fig. 1. Dressing of volunteer after removal of the cell itself, without cleaning.
2. Feeding by the box method.

PLATE 27. TRENCH FEVER.

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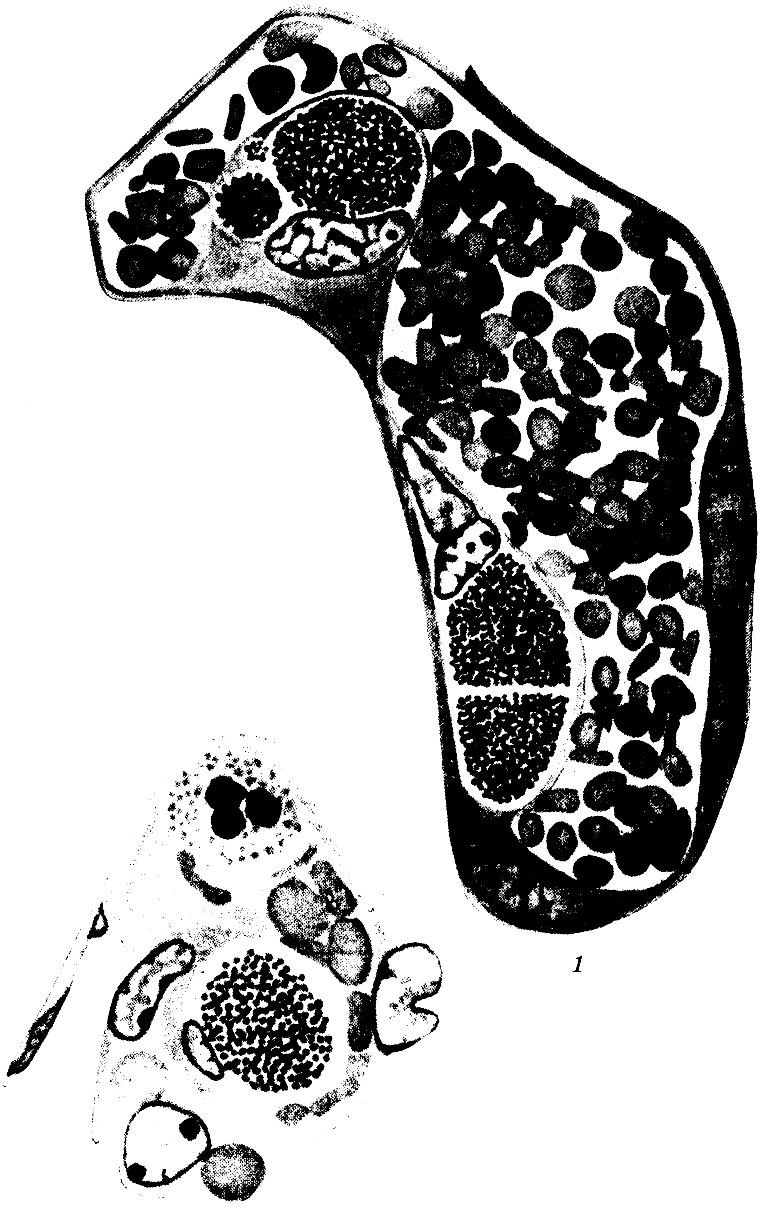

Fig. 1. Kidney showing large blood vessel with engorged endothelial cells.
2. Spleen showing swollen endothelial cell.

PLATE 28. HEARTWATER.

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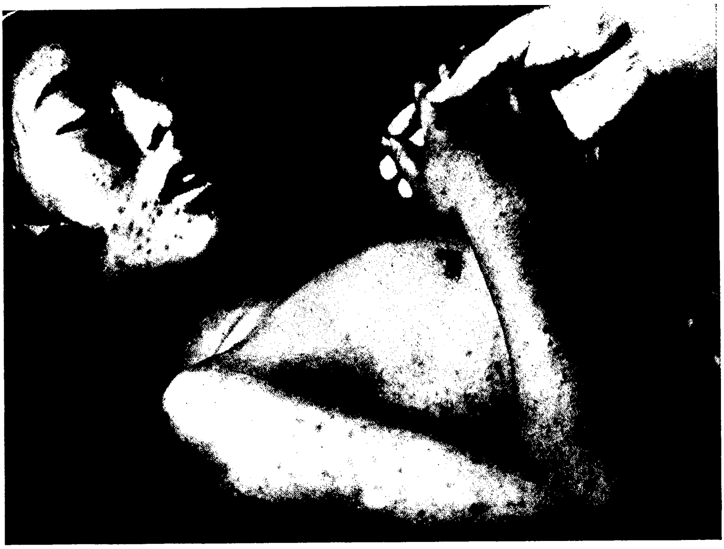

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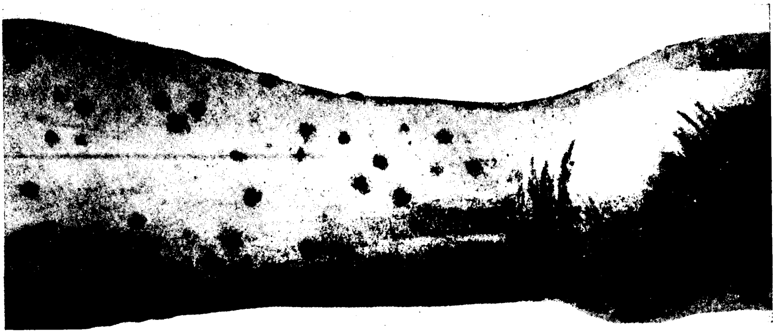

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Fig. 1. Eruption in a woman of 22, six days after onset. 2. Sketch of eruption on the arm of a man of 30.

PLATE 29. TSUTSUGAMUSHI FEVER.

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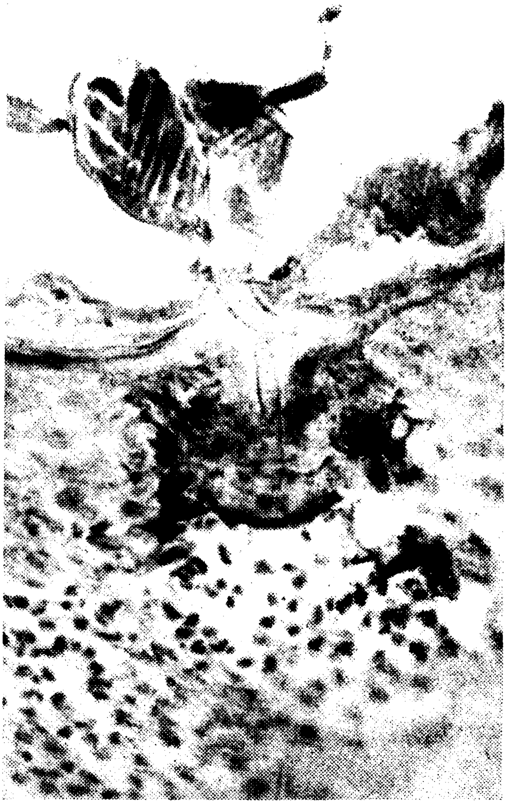

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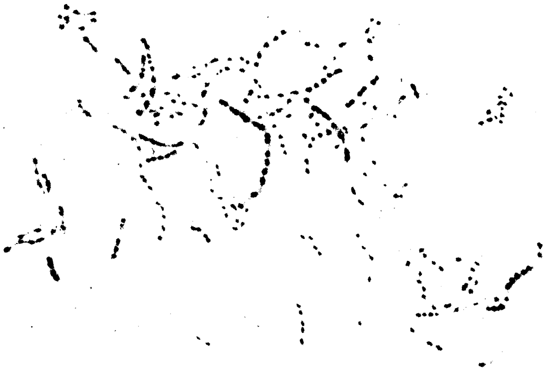

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Fig. 1. Human case. Bite lesion with the akamushi in situ. 2. Microörganisms cultivated from animals infected with tsutsugamushi virus.

PLATE 30. TSUTSUGAMUSHI FEVER.

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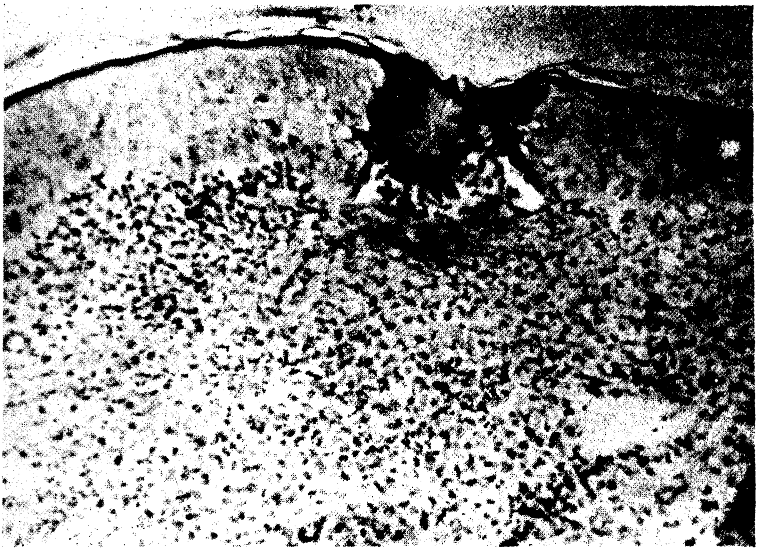

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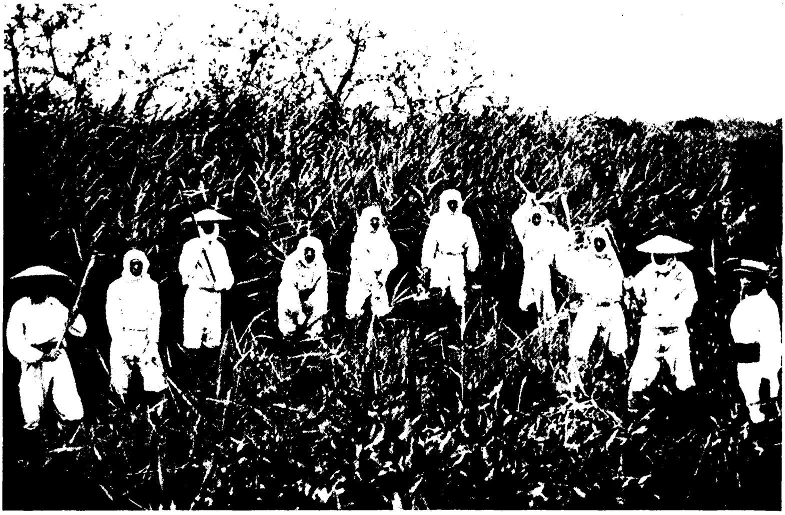

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Fig. 1. Tsutsugamushi fever (human case). 2. Men, protected by vermin-proof suits, catching field voles.

PLATE 31.

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CHAPTER XI

FILTERABLE VIRUS DISEASES OF FOWLS

LEUKEMIA OF CHICKENS

Definition.—Leukemia of chickens is an infectious disease of chickens that affects the blood-forming organs. It is characterized by a marked increase of the myeloid tissue in the liver and atrophy of the tissues of the bone marrow. There may be a diminished number of red blood cells and an increase of white corpuscles in the circulating blood.

History.—Ellermann and Bang(1) first recognized and studied this disease. The leukemia described in chickens by Moore(2) in 1896 was apparently due to *B. sanguinarium* and represented an acute infectious disease with a hyperleucocytosis. Hirschfeld and Jacob(3) had made a clinical study of leukemia in chickens in 1907, and their work was further extended by Skiba(4) in 1909. It was thought by Burckhardt(5) in 1912 that chicken leukemia was merely a chronic and progressive form of avian tuberculosis. The disease was first described in Denmark and has also been reported in parts of Germany. (Eber(6) and Göhre.(7))

The virus of chicken leukemia.—Very little is known concerning the virus of this disease. It was demonstrated by Ellermann and Bang that the cell-free filtrates from organ emulsions are capable of transmitting the disease and for this reason it has been classified with the filterable virus diseases. However, Hirschfeld and Jacob, and Burckhardt were unable to demonstrate the filterability of this virus through Berkefeld filters. The virus is present in the spleen, liver, and bone marrow. Ellermann and Bang observed bodies resembling protozoans in the bone-marrow, while Hirschfeld and Jacob found in the tissues of infected fowls a long bacillus, which upon injection into healthy chickens caused a mild anæmia associated with lymphocytosis.

The disease may be transmitted to chickens by intravenous or intraperitoneal injections of infected organs. Subcutaneous injections are negative. Fowls other than chickens are not susceptible. Guinea pigs are not susceptible to the virus.

Incubation period.—The period of incubation varies considerably and may range from one to two months. Only about 40 per cent of inoculated fowls become affected, and only about one-half of these develop a typical leukemia while the others show a pseudoleukemia.

Symptoms.—Skiba regards leukemia in chickens as a disease entirely different from leukemia in man. It has already been mentioned that Burckhardt considers the disease as a slow-developing and progressive tuberculosis. This author considers the large mononuclear cells as advanced stages of the erythrocytes. The striking changes found in affected fowls is the marked enlargement of the liver and spleen. Whatever the nature of the virus there is undoubtedly produced a great increase in the development of white blood cells in the bone-marrow, liver, and spleen, and frequently in other organs. In some cases lymphatic nodules have been described in the blood-forming organs which develop into lymphomas, while in other cases the accumulation of lymphoid cells in the capillaries is so great that it leads to occlusion of the vessels. Rarely tumors consisting of myelocytes appear in different parts of the body, and in these cases there is no leukemic alteration of the blood. These cases are similar to true myeloblastic leukemia with leucostasis. Other cases may show only a leucostasis in the liver and bone-marrow with an infiltration of the splenic pulp, diminished formation of red blood cells, and increased mononuclear leucocytes with few myelocytes in the blood. The disease may last for several months but usually runs its course in one to two weeks. Spontaneous recoveries are exceedingly rare, and death may result at any time during the disease.

Fowls susceptible to leukemia.—The disease is limited to chickens.

Immunity in chicken leukemia.—The fact that only about 40 per cent of inoculated fowls develop symptoms of the disease indicates that a high grade of natural immunity exists. However, this is all that can be said on the subject of immunity in this condition. It is not definitely known if one attack of the disease produces an immunity because so few recoveries have been noted.

Pathology.—The enlargement of the liver and spleen has been referred to. The liver has been known to weigh 300 grams in cases described by Ellermann. The outer surface and the cut surface of the liver present white flecks about the size of small peas. These changes may also be noted in the kidney, while the spleen is enlarged and red in color. Small lymphomas are also found in the skin and liver in some cases. The color index of the blood is usually high, being over 1 or 1.5. During the course of the disease severe bleedings may occur as a result of the hæmorrhagic diathesis. This has been noted in wounds of the comb. The leukemic picture in the blood develops suddenly and may not be noted during the life of the bird. The white blood cells are increased from 30,000 to 600,000. The proportion between the white blood cells and the red blood cells may be as high as 1: 250. A typical blood picture is as follows: Polynuclear cells, 4 per cent (1 to 10 per cent) ; transition cells, 51 per cent (40 to 60 per cent) ; myelocytes, 19 per cent (10 to 20 per cent) ; lymphocytes, 26 per cent. In one case Ellermann found 91 per cent to be lymphocytes. This is known as the lymphatic form of the disease.

Control measures.—Isolation of infected fowls and quarantine of healthy fowls brought into an infected area are indicated, though such measures frequently, because of the long period of incubation and duration of the disease in some fowls, do not at once terminate the spread of the disease. Arsenic and Röntgen-ray treatments have been tried, the former with recovery in one case which of course means little, the latter without effect on the course of the disease.

BIBLIOGRAPHY

1. Ellermann and Bang, Centralbal. f. Bakt. 46 (1908) 595 (lit.).
2. Moore, Bur. Animal Indus. Rpt. (1905) 185.
3. Hirschfeld and Jacob, Z. f. kl. Med. 69 (1909) 1 (lit.).
4. Skiba, D. t. W. (1909) 405 (lit.).
5. Burckhardt, Zeit. f. Imm. 14 (1912) 544.
6. Eber, Leipz. B. 1907–08) 54.
7. Göhre, S. B. (1909) 83.

OTHER REFERENCE

Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Eng. ed., Mohler and Eichhorn. Alexander Eger, Chicago 3 (1926).

FOWL POX AND AVIAN DIPHTHERIA

Chicken pox and Epithelioma contagiosum

Definition.—In the past avian diphtheria (epithelioma contagiosum) has been considered a contagious, epizoötic disease of fowls, characterized by diphtheritic pseudomembranes on the mucous membranes of the head.

Fowl pox (chicken pox) has been considered a contagious, epizoötic disease of fowls in which epithelial nodules appear upon the skin, especially on the comb and wattles, and in some cases, superimposed upon this picture, pseudodiphtheritic membranes appear upon the mucous membranes of the head.

The occurrence of a diphtheritic condition in both of these conditions led investigators to consider the possibility that perhaps they were dealing with only one disease and that the disease manifests itself in these two forms. Other investigators have pointed out the similarity of the lesions of fowl pox to the lesions of molluscum in man.

Since the question of identity of these diseases has not been fully settled, but because strong experimental evidence has been advanced to prove their unity, both forms of the disease (or both diseases) will be described in this review.

History.—Since the early work of Bollinger (1) in 1873 and of Rivolta (2) in 1881, avian diphtheria and fowl pox have been thought to be due to protozoans. In the case of avian diphtheria the flagellate *Cercomonas gallinæ* was thought to be the cause, while in pox gregarines were thought to be the etiological agent. In 1884 Loeffler (3) in his studies on diphtheria in pigeons isolated a bacillus which he thought was the cause of the disease and which he named *B. diphtheriæ columbarum.* Other investigators of the time found a similar organism in diphtheria of chickens. In 1894 Loir and Ducloux (4) discribed an ovoid bacillus as the cause of the disease. Müller (5) in 1906 reported cases in which he found a corynebacillus which was similar to the human diphtheria organism. As a result of these varied discoveries the belief became prevalent that avian diphtheria might be due to two or more organisms.

In 1902–03 Marx and Sticker (6) demonstrated that the virus of fowl pox was filterable through a porcelain filter. This was soon confirmed by Borrel, (7) Lowenthal, (8) and Burnet. (9)

Following these experiments, which demonstrated the filterability of the fowl-pox virus, great interest was manifested in the subject and experimental work was quickly advanced leading to grave doubts that the etiology of the two diseases was different.

In 1908 Carnwatch (10) produced in fowls a diphtheritic condition from pure fowl-pox material and also demonstrated that the diphtheritic material from his infected fowls produced chicken pox. These results were doubted by Bordet and Fally (11) but were confirmed by Schmidt,(12) Uhlenhuth and Manteufel,(13) and Rátz.(14) However, Bordet has contended that chicken diphtheria, in some cases, differs from chicken pox and is caused by a different agent.

Writing in 1926, Findlay and Ludford(15) state that in their opinion fowl pox has nothing in common with epithelioma contagiosum or with moluscum in man. On the other hand, they believe that the virus of fowl pox, if not identical, is closely related to the virus of vaccinia and their experiments are based upon cytological studies. The virus used in their experiments was obtained from a spontaneous epidemic of fowl pox.

Distribution.—Both fowl pox and avian diphtheria occur in various parts of the world. Both conditions have been reported in sections of the United States and in Europe and Asia. In Italy and Tunis the diphtheritic condition occurs most frequently and with greater virulence.

Incubation period.—In both conditions the incubation period is usually from six to twelve days. Slight variations from this may occur in different epidemics.

Symptoms.—The clinical picture varies with the form of the disease. In some epidemics the affection may involve only the skin or the mucous membranes, while in other epidemics both are involved.

When the skin alone is involved the condition is spoken of as chicken pox. In this form of the disease the skin of the head is first involved. At first a fine, branlike, gray deposit develops on the comb, ears, and wattles, and on parts of the body where the feathers are absent. These initial lesions soon develop into small nodules. These are at first reddish gray but later become grayish yellow. Later they become brown, dry, and firm, and their surface is warty. They contain horny or fatty degenerated epithelial cells. They may coalesce and bleed, and thick scabs may form over their surface. The margins of the nasal orifices and the eyelids become thickened, and the eyes remain shut. Should the conjunctiva become involved a panophthalmitis may develop. Nodules may also develop on the mucous membranes of the mouth and throat or on other parts of the body. When the lesions are confined to the skin the general condition remains good, but when widely disseminated marked loss in weight results. Aberrant forms of the disease are said to occur in which there is a marked febrile reaction and the lesions develop into pustules.

In the diphtheritic form there is usually no marked general disturbance. The condition usually begins in the mouth with the formation of a mucous membrane. This may result from scattered small yellowish-white areas or the first changes in the mucous membrane may be a change in color to a dark red which is soon followed by a gray deposit which leads to membrane formation. The membranes are usually adherent and leave a bleeding surface when they are torn away. The surface beneath is red and finely granulated.

The tissue surrounding the membrane is swollen and œdematous and may become membranous later in the course of the disease. The inflammatory process may extend to the larynx and on to the trachea and bronchi. Respiration and swallowing become very difficult, and inspiration is characterized by a whistling noise. Finally the appetite is lost, the animal can neither eat nor drink, and must be fed by hand.

The nasal cavity becomes involved, and there is a serous discharge which soon becomes mucopurulent. The lacrimal canal becomes blocked, the whole head becomes swollen, and the eyes shut. Later both the sclera and the cornea may be involved in the process. Also in the later stages of the disease the intestinal tract may become affected and there is diarrhœa which is sometimes streaked with blood and pus.

In some cases both chicken pox and the diphtheritic process are present at the same time or at different stages of the disease. This has been called the mixed form. In such cases the symptoms of both forms of the disease are present.

The disease is most acute as it occurs in Algeria and Cuba. In acute cases the animals undergo rapid exhaustion and die within a short time.

Where the disease is confined to the skin the course is usually favorable and runs about three to five weeks. In few cases the disease is prolonged for several months. The diphtheritic condition is less favorable, the mortality being from 50 to 70 per cent. The acute form mentioned above usually ends fatally within four to eight days.

Fowls susceptible to fowl pox and avian diphtheria.—Both conditions occur in chickens, turkeys, pheasants, peacocks, pigeons, and less commonly in water fowls.

The virus of fowl pox and avian diphtheria.—Reference has already been made to the original ideas of early workers that the cause of fowl pox and avian diphtheria was a protozoan. These bodies, then spoken of as gregarine parasites, are now known to represent cell inclusions. While the bacterial forms which have been described cannot be entirely dismissed from consideration, there seems to be a general agreement that they may be considered as secondary invaders or that they are the cause of disease processes which closely resemble avian diphtheria and for that reason have resulted in much confusion regarding the etiological agents concerned in these two conditions.

It is known, for example, that Berkefeld filtrates of epithelioma masses or of the diphtheritic membranes produce in fowls epitheliomas on the skin as well as diphtheritic membranes on the mucous membranes of the mouth. For this reason it seems to be well established that both conditions are caused by filterable agents. The inclusion bodies or "chicken pox bodies" that are found in the epithelial cells have been designated Chlamydozoa by Prowazek,(16) and Strongyloplasma by Lipschütz.(17) Emulsions of epitheliomas as well as the filtrates from them, contain small, spherical, nonmotile bodies, 0.25 micron in size, that stain with Giemsa's, with Loeffler's flagella stain, and with Ziehl's fuchsin. Prowazek believes these small bodies may penetrate the epithelial cells and there produce reaction products which result in the so-called cellular inclosures, or pox bodies. The theory has also been advanced that the pox bodies are thrown off by the nucleus of the cell and that the disease is due to a toxin thrown off by the epithelial cell.

Bordet * has reported the cultivation of small granules which measure about 0.2 micron, from diphtheritic material, on his blood-glycerin-potato agar. With these cultures he produced diphtheritic membranes in chickens, but his virus did not produce epithelioma on the skin. These results were not confirmed by Lipschütz or by Burnet.

The virus as contained in the epithelial nodules is very resistant to drying and to the action of the sunlight. Heating to 60° C. destroys it in about three hours, and freezing to —12° C. destroys it in about five weeks. One per cent phenol has no effect upon the virus. The virus is preserved in glycerin for an indefinite length of time.

Experimental pox lesions are produced on the skin by rubbing the material from pulverized nodules into the skin of healthy animals. The incubation period in this form of infection is

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* Doctor Bordet informed the author in August, 1928, in a personal interview that he now believes the microbe he was working with was the mocrobe of coryza contagiosa and not the incitant of fowl diphtheria as he previously thought.

about five or six days. Filtrates of such material also produce lesions but the incubation period is longer, usually eight to ten days. Pox lesions may also be produced upon the skin following intravenous injection of infected material. Burnet produced lesions on the skin by feeding scabs from infected animals. The virus is present in the blood stream early in the course of the disease since lesions have been produced by employing liver for the purpose of infection.

The virus of chicken pox and the virus of cow pox have several things in common. Both occur in the skin lesions, both are filterable, both are dermotropic and resistant to glycerin. Experiments have been advanced to prove that vaccine virus produces a local immunity in chickens to chicken-pox virus, but the work of Toth indicates that this is not true since vaccine virus, according to his experiments, immunizes only to vaccine virus and not to chicken-pox virus.

Close relation between avian diphtheria and diphtheria in man has been said to exist. Loir and Ducloux have found the same organism in the pseudomembrane from a child suffering with diphtheria that they found in the membranes of fowls; and the pesudodiphtheria bacillus, which is believed to be an avirulent form of the true diphtheria bacillus, has been found in birds by Gratia and Lienaux.(18) However, even though there may be a close relation between some of these organisms as they are found in fowls and the true diphtheria bacillus found in man, there is no case on record of the transmission of diphtheria from man to animals or vice versa.

It is possible to produce a diphtheritic inflammation on the tracheal mucous membranes in pigeons and chickens with true diphtheria toxin, but the condition so produced is not clinically the same picture as natural infection with the virus of avian diphtheria. Pox lesions have not been produced with such toxin.

Streit(19) in 1904 described the "Roupbacillus" as the causative organism in an affection of fowls which occurs in America and is known as American roup. This disease is clinically identical with chicken diphtheria and the roupbacillus is very similar to *B. pyocyaneus*.

In view of the various bacterial forms which have been described for this disease, several explanations present themselves. First, the bacterial forms that have been described that include the Klebs-Loeffler bacillus, the bacillus diphtheritica columbarum, the roupbacillus, the chicken-diphtheria bacillus of Müller, and the colonlike bacillus of Hausser,(20) all may represent secondary invaders which aggravate the condition by lowering the resistance of animals, and themselves produce pathology which confuses the true picture of the disease. An analogy of this may be found in hog cholera where *B. suipestifer* frequently accompanies the hog-cholera virus. Second, these bacteria may alone produce conditions in susceptible fowls which closely simulate true fowl diphtheria. Finally, one of them, such as the organism described by Bordet, may in time be proved to be the actual virus causing the disease. This is suggested on account of its minute size and on what is definitely known concerning the filterability of the virus of chicken pox and of chicken diphtheria.

Immunity in chicken pox and chicken diphtheria.—Carnwath, and Uhlenhuth and Manteufel have demonstrated that a skin immunity to chicken-pox virus can be produced by producing an affection of the mucous membrane with diphtheria material. This was confirmed by Sigwart.(21) Haring and Kofoid(22) are said to have produced immunity in chickens with pox virus against diphtheria. The relation of vaccine virus to chicken-pox virus has already been mentioned. One attack of chicken pox or fowl diphtheria produces a lasting immunity. Chickens may be immunized successfully by vaccination with pox material on the scarified skin. Serum from highly immunized fowls possesses no preventive or curative value. Manteufel has produced immunity in chickens by intravenous injection of lymph vaccine, and such protection lasts about two years.

Pathology.—The changes found after death in fowls which have died of the mixed form of the disease are chiefly in the skin, the mucous membranes of the upper respiratory passages, the mucous membranes of the trachea and bronchi, œdema of the lungs, and fibrinous membranes on the serous coats. The spleen and liver may show fine yellowish spots and the intestinal mucosa is frequently the sight of inflammatory changes and small extravasations of blood. The changes in the eye depend entirely upon the extent of the involvement of this organ.

Control measures.—Isolation of diseased birds is the chief method of control and prevention. Preventive inoculation may be employed if the epidemic warrants this procedure.

BIBLIOGRAPHY

1. BOLLINGER, Virch. Arch. 58 (1873) 349.
2. RIVOLTA (1881). Ref. in Rivolta e Delprato's "L'ornitojatria" Pisa.
3. LÖEFFLER, Mitt. d. G.-A. 2 (1884) 421.
4. LOIR and DUCLOUX, A. P. 8 (1894) 599.

5. Müller, Centralbl. f. Bakt. 41 (1906) 621 (lit.).
6. Marx and Sticker, D. m. W. (1902) 893; 79 (1903).
7. Borrel, Ann. de l'Inst. Past. 17 (1904) 81.
8. Lowenthal, Deut. med. Woch. 32 (1906) 678.
9. Burnet, Ann. de l'Inst. Past. 20 (1906) 742.
10. Carnwath, Arb. a. d. kais Gesundheitsamte 27 (1908) 388.
11. Bordet and Fally, A. P. 24 (1910) 563.
    Bordet, Ann. vet. (1907) 494.
12. Schmidt, Centralbl. f. Bakt. 52 (1909) 200.
13. Uhlenhuth and Manteufel, Arb. d. G.-A 33 (1910) 288 (lit.).
14. Rátz, A. L. (1911) 293.
15. Findlay and Ludford, Brit. Journ. Exp. Path. 7 (1926) 256.
16. Prowazek, Arch. f. Protistenk 10 (1907).
17. Lipschütz, Centralbl. f. Bakt. 46 (1908) 609.
18. Gratia and Liénaux, Ann. (1898) 401.
19. Streit, Zeit. f. Hyg. 46 (1904) 407.
20. Hauser, Centralbl. f. Bakt. 48 (1909) 535.
21. Sigwart, Centralbl. f. Bakt. 56 (1910) 428.
22. Haring and Kofoi, quoted from Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Am. ed. Trans. by Mohler and Eichhorn 1 (1926).

OTHER REFERENCES

Kampmann, Hirschburg, and Lange, Centralbl. f. Bakt. 34 (1903) 214.
Lipschütz, Prowazek's Handb. d. Path. Prot. (1911) (lit.).
Kempa, Diss. Bern. (1909).
Apolent, Virch. Arch. 174 (1903) 86.
Benda, Centralbl. f. Allg. Path. 8 (1897) 862.
Lusena, Lo Sperimentale 79 (1925) 969.
Michaelis, Zeit. f. Krebsforsch. 1 (1903) 105.
Reischuer, Centralbl. f. Bakt. 40 (1906) 356, 474, 653.
Sanfelice, Zeit. f. Hyg. u. Infekt. 26 (1897) 298.

FOWL PLAGUE

PESTIS GALLINARUM, TYPHUS EXSUDATIVUS GALLINARUM; PERITONITIS EPIZOÖTICA, KYANALOPHIAEA; PESTE AVIAIRE (FRENCH); GEFLÜGELPEST (GERMAN)

Definition.—Fowl plague, or fowl pest, is an acute, infectious, contagious affection of fowls characterized by petechial hæmorrhages disseminated throughout the body and by symptoms which strongly resemble those of fowl cholera.

History.—Fowl pest was first described in 1878 but has appeared in serious epidemics only since 1894. In that year it appeared in northern Italy and spread to Germany. Later it spread to Tyrol, France, and Belgium, and to the United States in 1924.

Distribution.—Fowl plague is found in the European countries Italy, Germany, France, and Belgium. It has been endemic in the United States, where the disease differs from the European form only in the species of fowl most affected. It did not appear in the United States until 1924, though it had been reported prior to that date in South America in Argentine and Brazil.

Incubation period.—The incubation period in natural infection ranges from two to five days and in some cases may be prolonged to the seventh day. In artificial infection death frequently results within thirty-six to forty-eight hours.

Symptoms.—The affection usually begins with depression and a loss of appetite. Later the animal become oblivious to its surroundings, is stuporous, dull, and sleepy. The comb and wattles become blackish red (kyanolopiæa), the skin becomes scaly, the eyes closed, and the conjunctiva congested, swollen, and inflamed. There is a mucous secretion from the nose and throat, and hæmorrhagic spots may be noted on the mucous membranes. There may be an associated diarrhœa, and the fever may reach 44° C. or more. The disease usually runs its course in two to four days but may last for a week or more. It is usually fatal.

Aberrant forms of the disease have been described in which diphtheritic membranes are formed in the throat, and in other cases there are pronounced nervous symptoms which result in paralysis.

Fowls susceptible to fowl-pest virus.—Chickens, turkeys, pheasants, sparrows, blackbirds, sparrow hawks, owls, and parrots are susceptible to fowl plague. It is said that water fowls and pigeons are resistant to natural infection but are sometimes susceptible to artificial infection. The disease as it occurs in the United States is apparently not pathogenic for ducks, geese, or pigeons. Rosenthal(1) and Schiffmann,(2) however, claim to have established the disease in geese.

The virus of fowl pest.—In 1901 the virus of fowl plague was found by Centanni and Savonuzzi to pass through a porcelain filter. This has been confirmed repeatedly since then, and there is no reason to suppose that the etiological agent of this disease is any other than a filterable virus. The virus is present in the blood stream early in the course of the disease. It is also present in the nasal secretions, in the bile, and in the exudate of the serous cavities. Filtrates of these fluids are also virulent.

Landsteiner(3) states that the virus is adherent to the blood cells and can be cultivated up to the tenth generation by placing defibrinated blood upon agar plates. Rosenthal, Kleine,(4) and Schiffmann have all described small ring-shaped bodies inside and outside the brain cells. These bodies may also be round or oval and their true nature has not been determined. Prowazek(5) did not confirm these findings but describes in the brain tissues small dumb-bell-shaped forms which measure about 1 to 1.5 microns in size. These forms frequently lie close to the red blood cells, and are also demonstrable in filtrates of material known to contain the virus. Prowazek classes these small bodies with the Chalamydozoa, their protozoan nature being also indicated by their resistance to glycerin. The virus is believed to be smaller than hæmoglobin molecules since it passes through Bechhold's ultrafilters that do not allow hæmoglobin to pass.

The virus is destroyed almost immediately by heating at 65° C. It will resist 50° C. for nearly thirty minutes. The virus will remain alive in dried tissue for nearly a year, and is preserved by glycerin for over a year (Maue(6)).

Immunity in fowl plague.—One attack of the disease confers an immunity to subsequent infection. Kraus and Schiffmann(7) report that dried spinal cord from infected geese will protect young geese. Geese may be immunized also with virulent spinal cord from chickens against a fatal subdural infection.

Pathology.—To Freese(8) we owe most for the pathological anatomy of this disease. In the very acute forms of the disease there may be few changes to note at autopsy. Changes most frequently found in animals dead of the disease are hæmorrhages on the inner surface of the breast bone, on the visceral layer of the pericardium, on the peritoneum, in the fat tissue of the gizzard, and other mucous membranes. The kidney and spleen may be hyperæmic, and there may be a small amount of cloudy fluid in the pericardial sac.

There is a catarrhal condition of the respiratory tract and of the gastrointestinal tract, and frequently small hæmorrhages are found in the lungs.

Control measures for fowl plague.—Control measures consist in isolation of affected birds, careful selection of new birds which are to be placed with healthy flocks, quarantine of new birds coming in, and careful disposal of the remains of birds that have died of the disease. The usual care of the premises is, of course, indicated.

BIBLIOGRAPHY

1. ROSENTHAL, Centralbl. f. Bakt. 40 (1905) 204.
2. SCHIFFMANN, Wein. klin. Woch. 19 (1906) 1347; Centralbl. f. Bakt. 45 (1907) 393.
3. LANDSTEINER, Centralbl. f. Bakt. 38 (1906) 540.
4. KLEINE, Zeit. f. Hyg., u. Infekt. 51 (1905–06) 177.
5. PROWAZEK, M. m. W. (1908) 165, 1016.
6. MAUE, Arb. d. G.-A. 21 (1904) 537.
7. KRAUS and SCHIFFMANN, Centralbl. f. Bakt. 43 (1907) 825.
8. FREESE, D. t. W. (1908) 173 (lit.).

OTHER REFERENCES

KELSER, Manual of Veterinary Bacteriology. Williams and Wilkins, Baltimore (1927).

KRAUS and DOERR, Centralbl. f. Bakt. 46 (1908) 709.

MARCHOUX, C. R. 147 (1908) 357; Soc. biol. 68 (1910) 360.

HALÁSZ, Közl. 10 (1912) 42.

HUTYRA and MAREK, Pathology and Therapeutics of the Diseases of Domestic Animals, 3d Eng. ed. by Mohler and Eichhorn. Alexander Eger, Chicago 1 (1926).

ROUS CHICKEN SARCOMATA

In 1910 Rous(1) first described a sarcoma of the common fowl which is transmissible to other fowls. This sarcoma proved to be a spindle-celled tumor both infiltrative and destructive. In his first experiments Rous satisfied himself that the tumor was transmissible to other fowls of a closely related stock, and he propagated the tumor into its fourth generation. Later, in 1911, Rous was able to show that this tumor of the fowl was extremely malignant and possessed a marked tendency to widespread metastasis. Furthermore, the tumor could be transmitted to barred Plymouth Rock fowls not only by inoculation with the tumor material itself but also with Berkefeld filtrates of the tumor emulsion. Also it was discovered that the tumor could be transmitted to susceptible fowls with dead cells, killed by desiccation or by 50 per cent glycerol. In this respect Rous chicken sarcoma No. I differs from all mammalian tumors. Mammalian tumors have only been transmitted by living cells.

In 1912, Rous, Murphy, and Tytler reported a second tumor, an osteochondrosarcoma, of fowls which they believed to be due also to a filterable agent. At the same time a second spindle-celled sarcoma differing from the first tumor of this type described by Rous was also mentioned. This tumor was also thought to be caused by a filterable agent. These three tumors have been designated and described in the literature as 1, spindle-celled chicken sarcoma No. I; 2, osteochondrosarcoma of chickens, tumor No. VII; and 3, spindle-celled chicken sarcoma with blood sinuses, tumor No. XVIII. The last was fully described in 1914.

All of these three tumors have been demonstrated by Rous and his associates to be transmissible to other fowls. Berkefeld filtrates of each tumor are capable of inducing typical tumor formation when inoculated into susceptible fowls. All three tumors then have been regarded as being caused by a filterable agent.

Chicken sarcoma No. I, spindle-celled, can be transferred from chicken to chicken by tumor emulsion, filtrate of tumor emulsion, or by tumor cells which had been killed by drying or by 50 per cent glycerol. This tumor usually kills its host within twenty-eight days. It is prone to metastasize and upon passage increases in malignancy. It cannot be transmitted to mammals or to any species of bird other than the chicken. When first studied sarcoma No. I was highly specific for one closely related strain of chickens, and while it still grows more freely in Plymouth Rock hens, it is also transmissible to other breeds of chickens.

Chicken osteochondrosarcoma No. VII when first studied was apparently benign but proved malignant after repeated passage. It is an entirely different tumor from tumor No. I. This tumor is also said to be transmitted with either filtrates of the tumor emulsion or with dead tumor cells.

Chicken sarcoma No. XVIII, spindle-celled with blood sinuses, can be transmitted to other fowls with Berkefeld filtrates of the tumor emulsion or with the living tumor cells. It cannot be transmitted with the dried tumor material.

Numerous spontaneous tumors of chickens have been described, but many of these have resisted transplantation. These three tumors which have been described by Rous and his associates are all transmissible; the latter two, No. VII and No. XVIII, represent only two of about thirty tumors of chickens studied by these authors.

Early in his experiment Rous found that the addition of a small amount of infusorial earth, Kieselguhr, to his filtrates apparently aided in the development of the tumor. However, this is not essential, as shown by protocols where tumors have developed in the presence of the filtrate alone and in the absence of this substance.

Each tumor possesses its own characteristic histologic composition suggested by its name. For further details of histology the reader is referred to the original papers describing these growths.

For many years the chicken sarcomata of Rous were regarded as tumor formations caused by filterable agents, possibly filterable viruses. Since the early days of medical science there have been those who regard all forms of malignant growth (cancers and sarcomata) as being due to parasites (bacteria, protozoans, fungi, etc.). This has been known as the parasitic theory of the origin of cancer and other malignant neoplasms. Opposed to this concept have been those who consider malignancy as being produced by a variety of other causes, such as chronic irritation, diet, chemical agents, and hereditary factors. The contagiousness of malignant growths has long been a mooted question and even to-day remains unsettled. There are few, however, who believe in the contagiousness of these tumors.

The discovery by Rous and his associates of malignant tumors in chickens and the evidence adduced by them demonstrating that filterable agents were the causative factors led to much speculation. Could all malignant growths be due to filterable viruses? Are cancers and sarcomata in human beings caused by filterable viruses? What of all the statistics on heredity of malignancy in human beings, the theories of chronic irritation, both mechanical and chemical; what of the theories concerning diet and cancer; and of the heredity studies of Maud Slye on mouse tumors?

In 1925 Gye(2) and Barnard(3) gave new impetus to the study of the question in their reports on the origin of cancer. This work was based on the early reports of Rous on the chicken spindle-celled sarcoma No. I. The report of Gye was startling and still remains to be adequately confirmed by other investigators. This author claimed to have demonstrated that the Rous tumor No. I is due to a virus and further that he had succeeded in cultivating this virus in vitro. Also Gye reported that he was able to transmit mouse sarcoma 37/S with a cell-free filtrate; that rat tumor No. 9, a carcinoma, and Jansen's rat sarcoma, mouse carcinoma 63, and a human-breast carcinoma, all provide a "factor" which "can replace the virus of the Rous tumor in the genesis of a chicken sarcoma." He believes that the common "factor" in the last four tumors is certainly a virus. He also states that the virus "alone" is unable to bring about the malignant changes of a cell and that there must be an "adjuvant" substance, which he has termed the "specific factor" in order to induce the tumor formation. Gye states—

> These researches have led me to look upon cancer—using the term in its widest sense—as a specific disease caused by a virus (or group of viruses). Under experimental conditions the virus alone is ineffective; a second specific factor, obtained from tumor extracts, ruptures the cell defenses and enables the virus to infect. Under natural conditions continued "irritation" of tissues sets up a state under which infection can occur. The connection between the specific factor of a tumor and an irritant remains to be investigated. Some of the relatively unimportant "irritants" are known, such as coal tar, paraffin oils, etc. The virus probably lives and multiplies in the cell and provokes the cell to continue multiplication.

Gye claimed to have cultivated the virus of the Rous No. I tumor in a rabbit serum KCL broth, Hartley's broth, to which a fragment of chick embryo is added. He states, "the age of the embryo used has generally been 12 to 16 days." The primary cultures were incubated in anaërobic jars at 35 to 36° C. for four days, after which subcultures were prepared. He has assumed that, following tests of his cultures upon susceptible fowls, if growths are obtained with subcultures beyond the fifth passage multiplication of the virus has taken place. He estimates that each subculture represents at least a dilution of the original virus one thousand times and calculates that in the fifth passage multiplication of the virus must have taken place. He estimates that each subculture represents at least a dilution of the original virus one thousand times and calculates that the fifth subculture is a dilution of one thousand billion, or 10^{15}.

Barnard's contribution to the study by Gye of the origin of cancer consisted in photographing, by means of ultra-violet light, what is considered by these authors to be the actual virus of the Rous chicken sarcoma as well as other filterable viruses such as the virus of pleuropneumonia, mouse sarcoma 37/S, and human carcinoma. The "virus" has the appearance of extremely minute spheroids. Barnard states—

> Probably no single factor has hindered the cultivation of filterable viruses so much as the assumption that a culture in broth, for instance, must become opalescent, or that a growth on solid media must develop colonies easily visible to the naked eye. If as we think probable the spheroids are an essential part of the life-history of these organisms then, owing to their low refractive index, they would require to be present in impossibly great numbers to produce evident cloudiness.

The criteria assumed by Gye and Barnard for growth of the filterable virus do not seem severe enough to us. While most investigators will admit that a dilution of one thousand billion away from the initial inoculum is exceedingly high, yet it is conceivably possible that a few minute cells of the tumor may be carried over through each successive subculture. Furthermore, Barnard's photographs do not convince one that he is actually looking at photographs of the "virus." Indeed, Barnard himself states, "Unless considerable care is exercised the spheroids may easily be confused with other spherical bodies occurring in most organic fluids." It will be recalled that Novy found his rat virus active in 0.00000000001 of a cubic centimeter and conceivably a 1 to 10 dilution of this quantity in certain instances, at least, might have proved infective. (See Novy's rat virus, Chapter VII.)

Since the publications of Gye and Barnard numerous investigators have attempted to repeat their work. While many reports have been published, only a few will be mentioned. Mueller(4) for instance has been unable to duplicate the work of Gye and points out that uncontrollable local and individual variations may produce results in occasional chicks which simulate satisfactory experiments, but as he states, when viewed as a whole, mean nothing. Mueller concludes that—

> Because of the conflicting nature of results obtained by those who have undertaken to repeat the work, and on account of the difficulty of controlling all factors involved, we do not feel that it may be stated definitely that Gye's theory of the cause of cancer is wrong. On the other hand the theory apparently needs more evidence in its support if it is to receive further serious consideration.

Harkins, Schamberg, and Kolmer(5) have been unable to confirm the evidence of growth of the virus of the Rous chicken sarcoma in subcultures and find no evidence to support the theory that the so-called specific factor acts by reason of the presence of a chemical substance. These authors are inclined to the view that the specific factor is nothing more than a suspension of attenuated living virus which may or may not induce tumor formation, depending upon the susceptibility of the fowl.

The specific factor from the Rous sarcoma is obtained, according to Gye's method, by treating the filtrate with chloroform which is supposed to kill the virus. In Gye's experiments tumors were not produced following the injection of the virus alone or the specific factor alone. When the two were mixed, 0.5 cubic centimeter of each, tumors were always produced in about two weeks. Harkins, Schamberg, and Kolmer are inclined to doubt that the infectivity of the specific factor is destroyed when prepared according to Gye's method. Mueller also points out the difficulty of preparing suitable chloroform filtrates. He states that individual filtrates vary considerably and that the amount of chloroform employed is an important consideration. Murphy(6) has demonstrated that it is possible to reactivate chloroform filtrates by means of tumor cultures and states that these results are due to an acceleration of a partially destroyed agent by means of some substance common to rapidly growing tissues such as tumor, embryo, or placenta.

Cori(7) has reported a similar experiment. Flu(8) states that reactivitation may be obtained not only with tumor cultures and embryo culture, but also with similarly prepared material of liver and kidney tissues from chickens and guinea pigs. Harkins, Schamberg, Kolmer, and Kast, however, find that when sufficient chloroform is added to sarcoma filtrates so that the material when injected into chickens will not produce tumor growth, the infectiousness of the filtrate is not altered when tumor culture is added to it. According to Flu there are three weak points in Gye's theory on the origin of cancer. Briefly, these may be stated as follows: First, it is not at all certain that chloroform destroys all the virus in the tumor extract. Second, the extension of Gye's virus etiology to include mouse sarcoma. In Flu's experiments he failed to induce tumor formation in sixty mice by injections of cell-free filtrate or with unfiltered supernatant bouillon over pieces of this tumor. Third, the Rous chicken sarcoma, upon which Gye bases his theory, has been regarded by some competent pathologists as a chronic infectious granuloma and not a true sarcoma in any sense.

Simon and Beck(9) have also presented evidence that tumors are not produced by combining embryonic tissue cultures and chloroformed tumor extracts when the filtrates had been definitely inactivated. These authors state that the chloroform treatment of the tumor extract does not lead to a complete inactivation of the contained "virus."

Finally, Nakahara(10) has shown that the cells of the Rous chicken sarcoma are often filterable through the coarse and medium Berkefeld filters and that a small number of these cells can be demonstrated in a proportion of the filtrates. This author has shown that this tumor may be transmitted by an injection of a very small number of washed sarcoma cells and the addition of filtrate to a large number of cells produces no more effect than does so much distilled water. Nakahara concludes that the transmission of the Rous chicken sarcoma may not be independent from the actual transplantation of viable sarcoma cells. He further points out, in conection with desiccation and glycerination of the Rous chicken sarcoma No. I, that some viable cells can be demonstrated when such tissue retains the power to produce sarcoma.

Studies such as have been described above on the etiology of malignant new growths are of prime importance to human medicine. Malignant diseases are among the most important of human ailments, and it has been hoped that the study of similar conditions in experimental animals might throw some light upon their etiology. Some authorities have been inclined to consider chronic irritation (whether mechanical, chemical, toxic, etc.) as always being the basic factor involved in the production of these growths, while others have continued to search for some specific cause in the nature of a living parasite, germ, or virus. It will be recalled that Fibiger(11) was able to produce cancer in the gastric mucosa of the rat by feeding cockroaches that he later found harbored a certain worm which he named *Spiroptera neoplastica.* In this case Fibiger believed the chronic irritation was produced by some toxic secretion from the parasite. It should be pointed out, however, that this irritant was incapable of producing any cancer other than the gastric cancer of the rat. It is, of course, common knowledge that cancers can be produced in rats by painting the skin with coal tar over prolonged periods of time, and it is a historic fact that sheep herders developed cancer of the skin as the result of carrying charcoal ovens next to their bodies over which they were accustomed to warm their hands during long vigils of work and exposure to the elements. While we are interested here chiefly in the filterable virus theory of the origin of neoplasms, it is of interest to comment on the "irritation" theory.

Recently Sturm and Murphy(12) have attempted to separate a causative agent from the cells of a tar sarcoma of the chicken. These authors were unsuccessful either with filtrates or desiccates, and they point out that this tumor "must stand as an exception in the chicken tumor group, in that it resembles the mammalian tumors in the failure to be transmitted by an agent separable from the living cell."

It is evident that the theory of Gye and Barnard on the origin of cancer has met with serious objection on the part of investigators thoroughly competent to evaluate the protocols that have been advanced in its favor and who have demonstrated conservatism in the interpretation of their own experimental work. While it is premature to condemn entirely the theory of Gye, we are inclined to agree with Mueller that more-convincing evidence must be presented in its favor if the "virus theory" is to receive further serious consideration.

BIBLIOGRAPHY

1. ROUS, Journ. Exp. Med. 12 (1910) 696; 13 (1911) 397.
   ROUS, MURPHY, and TYTLER, Journ. Am. Med. Assoc. 59 (1912) 1793.
   ROUS and LANGE, Journ. Exp. Med. 18 (1913) 651.
   ROUS, Journ. Exp. Med. 19 (1914) 570.
2. GYE, Lancet 2 (1925) 109.
   GYE, Brit. Med. Journ. 2 (1925) 291.
   GYE and ANDREWS, Brit. Journ. Exp. Path. 7 (1926) 81.
3. BARNARD, Lancet 2 (1925) 117.
4. MUELLER, Proc. Soc. Exp. Biol. & Med. 23 (8) (1926) 704; Journ. Exp. Med. 45 (1927) 243.
5. HARKINS, SCHAMBERG, KOLMER, and KAST, Journ. Cancer Res. 10 (1926) 66.
6. MURPHY, Journ. Am. Med. Assoc. 86 (1926) 1270.
7. CORI, Proc. Soc. Exp. Biol. & Med. 24 (1926) 65.
8. FLU, Centralbl. Bakt. (etc.) Abt. 1, Orig. 99 (1926) 332.
9. SIMON and BECK, Am. Journ. Hyg. 6 (1926) 659.
10. NAKAHARA, Sc. Rep. Govt. Inst. Infect. Dis., Tokyo 5 (1926) 287.
11. FIBIGER, Zeitschrift f. Krebsforschung 13: 217, 14: 295; Orbituary, Brit. Med. Journ. 1 (1928) 200.
12. STURM and MURPHY, Journ. Exp. Med. 47 (1928) 493.

NEUROLYMPHOMATOSIS GALLINARUM: FOWL PARALYSIS

Definition.—Neurolymphomatosis gallinarum (Pappenheimer, Dunn, and Cone) is an endemic disease of fowls which is characterized by an asymmetrical, partial, and progressive paralysis of the wings and both legs with occasional gray discoloration of the iris and blindness. In from 4 to 12.9 per cent of the cases lymphomata have been found that exceed the tumor rate for normal fowls. The cause of the disease is unknown.

History.—According to the recent studies of Pappenheimer, Dunn, and Cone(1) fowl paralysis was first described by Marek in 1907. Later important clinical studies of the disease were made by Kaupp in 1914 and reported by this author in 1921. The disease is said to occur in all parts of the United States and Canada. There is reason to believe that the same disease or one quite similar is also prevalent in Austria, Holland, Argentina, and perhaps other parts of the world.

Etiology.—The etiology of fowl paralysis is unknown. In the most recent comprehensive experimental work of Pappenheimer, Dunn, and Cone several possible theories of the cause of the disease are suggested. While these authors are very conservative in their deductions from their experimental work they seem to favor the theory that the cause of this disease is a living virus, presumably an ultravirus since no microörganism can be demonstrated in material from cases of the disease. Other possible causes such as a nutritional disorder, an intoxication, and a neoplastic process are given full consideration, but none of these seems probable. For these reasons this disease has, at least temporarily, been placed in the group of filterable virus diseases of fowls. In the experience of these authors not more than 25 per cent of birds inoculated with material from diseased animals develop the disease. It occurs in both sexes, and all common breeds of chickens may be affected. No relation has been found between paralysis and infestation with coccidia and intestinal worms.

Incubation period.—The precise incubation period in this disease is unknown. According to Pappenheimer, Dunn, and Cone the earliest clinical cases in their experimental material were ten weeks of age. Apparently there was only one case that developed symptoms as early as this. In the spontaneous cases studied by these authors clinical symptoms were observed at twelve weeks and the oldest case developed paralysis at fifteen months eighteen days. These authors state that the onset of paralysis may be very sudden. They have observed a complete loss of power in the legs in less than three hours. These observations indicate the age incidence of the disease, however, rather than the incubation period. In their chicken No. 4909, which was inoculated intramuscularly into the left leg with 0.2 cubic centimeter of suspension of cord and sciatic nerve from G. 1348 (infected fowl), April 16, 1926, histologic lesions characteristic of the disease were found two and one-half months later when the fowl died without showing previous symptoms. In another fowl injected with the same material upon the same day symptoms of paralysis developed in about four months following the inoculation.

Symptoms.—Drooping of a wing on one side, lack of coördination in walking, limping gait, and finally complete prostration are early manifestations of the disease. According to

Pappenheimer, Dunn, and Cone the limbs are not symmetrically affected even in advanced cases. The chickens usually lie upon the side that shows greater paralysis. In older cases muscular atrophy may become extreme. The paralysis is more often spastic than flaccid, and in some cases there may be clonic spasms. The duration of the disease is variable. In one of the ten early cases at the Storrs Experimental Station a completely prostrated fowl lived from October, 1922, until January, 1923, under careful feeding. Death, however, may occur suddenly even though there may be periods of transient improvement. Partial or complete blindness may occur in some cases. Lymphomata occur in a certain percentage of cases.

Susceptible fowls.—The disease is apparently limited to chickens. All common breeds are susceptible, and both sexes are affected in about the same proportion.

Immunity.—That there is apparently a high degree of natural immunity possessed by some fowls is indicated by several facts. First, only about 25 per cent of inoculated fowls develop the disease; second, the disease is so mild in some cases that the symptoms may be entirely overlooked; third, the spontaneous infection does not infect the entire flock but apparently is confined to only the more susceptible fowls.

Pathology.—To Pappenheimer, Dunn, and Cone belongs the credit for a comprehensive study of the anatomical changes produced in fowls by this disease. According to these authors—

> in the peripheral nerves, the essential feature is an intense infiltration of lymphoid, plasma cells, and large mono-nuclears. This is accompanied by a myelin degeneration in the more advanced lesions, but the cellular infiltrations appear to precede the degenerative changes. In the brain and cord and meninges, there are similar infiltrations predominantly perivascular. Infiltrations of the iris with lymphoid and plasma cells are found in the cases showing gross discoloration of the iris. Visceral lymphomata, originating in the ovary, are associated in a certain percentage of the cases. Evidence is presented in favor of the view that this association is not accidental and that the lymphomata are a manifestation of the disease.

Control measures.—As evidence of the infectiousness of this disease has been presented, isolation of infected birds and quarantine of healthy birds seem to be indicated.

May 8, 1928, Pappenheimer(2) sent the following personal communication to the author concerning further experiments that he had performed:

> Nine Spangled Hamburg 6-day chicks, were inoculated with 0.1 cubic centimeter of Berkefeld N filtrate (ground in sterile Tyrodes solution) of

cord and brain ganglia of a typical paralyzed fowl. Eleven of the same hatching received in addition 0.1 cubic centimeter of a freshly prepared section of embryo extract in the hope that this addition might activate the virus. The controls, sixteen in number, received the embroyonic extract alone. These controls were thought necessary to exclude the possibility that the particular embryonic extract used might carry the virus. The reason for choosing the Spangled Hamburg strain was that in our work at Storrs, we found this variety to be particularly susceptible.

The results of this experiment were entirely negative both as regards the transmissibility of the disease by the inoculation of the filtrate alone, or with the addition of the embryonic extract. Our controls also remained free of paralysis. While this is only a single experiment, it obviously offers no support for the virus hypothesis. One thing that we did learn from study of this series was the frequency with which perivascular lymphoid accumulations may be found in the brain cord and ganglia of apparently healthy normal fowls. In the brain they were present in over 30 per cent of the chicks and in the spinal cord in 13 per cent. I have reached the conclusion that the presence of these lymphoid foci cannot be taken as evidence of encephalomyelitis. Of course, their occurrence makes still more difficult the interpretation of the lesions.

BIBLIOGRAPHY

1. PAPPENHEIMER, DUNN, and CONE, Bull. Storrs Agricultural Experiment Station, Storrs, Conn. (December, 1926) (lit.).
2. PAPPENHEIMER, Personal communication (May 8, 1928).

PHILIPPINE FOWL DISEASE

What is believed to be a new disease of fowls recently appeared in the Philippines. The epizoötic began in September, 1927, and proved to be highly contagious. The first cases of the disease appeared in Manila. The infection spread rapidly over an area having a radius of about fifty miles with Manila as the center. By February 1, 1928, it had been estimated that at least fifty thousands fowls had succumbed as a result of this disease. Both males and females are affected, and fowls of all ages are susceptible. The disease has been prevalent in chickens, and a few cases have been noted in ducks and geese.

Symptoms.—The onset of the disease is sudden. Chickens at first show an indisposition to move about, preferring to sit or stand quietly in a secluded spot. Very early in the course of the disease there is a diarrhœa which gradually improves if the fowl is to recover. Gasping for air associated with jerking movements of the head downward and backward is a characteristic symptom. This is caused by large quantities of tenacious mucus which obstruct the posterior nares and pharynx. Many fowls die of suffocation early in the course of the disease. Excessive thirst is noted in many cases, and the crop is often filled with large quantities of water and mucus as well as foul-smelling gas in some instances.

Occasionally there is a bloody diarrhœa, but this is not a common symptom. The fowls usually die within one to seven days following the onset of symptoms. If the fowl is to recover from the disease the mucus in the nose and throat disappears, but there gradually develops a paralysis of the legs which may be either unilateral or bilateral. Of the fowls that recover from the acute symptoms and develop paralysis only a small percentage, about 5 per cent, fully recover from the paralysis. Paralyzed fowls may live for indefinite periods provided they are fed by hand and given water at regular intervals by a medicine dropper. Fully 99 per cent of fowls develop paralysis if they survive the acute stages of the disease.

The Philippine fowl disease is similar in some respects to other diseases known to occur in fowls. It is similar to infectious bronchitis of fowls in that there is the characteristic posture of the sick fowl, head extended, beak open, and a gasping for breath in both conditions. The paralysis somewhat resembles the chicken paralysis disease of fowls which has been described by Pappenheimer, Dunn, and Cone but is different from this disease in many of its other clinical manifestations. In the early stages the Philippine fowl disease resembles both infectious and nutritional roop. It is similar to fowl pest, especially in the per-acute cases which die within twenty-four hours. Fowl pest is transmitted to healthy fowls by minute quantities of blood, but this disease is not so transmitted.

Pathology.—Only the gross changes have been studied. The disease is essentially an infection of the alimentary tract. In all cases there is a periœsophageal inflammation that may involve the crop and extend throughout the entire length of the œsophagus. This inflammation is accompanied by hæmorrhages which vary in size from small petecheal hæmorrhages to large hæmmorrhagic streaks or extravasations of blood. The changes in the intestinal tract are hæmorrhagic in nature and vary greatly in degree. Petecheal hæmorrhages are also found on all the serous membranes. The eyes, nose, and throat are all filled with a tenacious glary mucus during the first days of the disease, and the crop frequently contains large quantities of this material. Histologic studies have been made only of the brain and cord taken from fowls during the stage of paralysis, but no definite changes have been found so far in the small amount of material that has been examined.

Etiology of the disease.—No bacterial incitant of the disease has been recovered from infected animals. The disease can be transmitted to healthy fowls by feeding the washed tissue of the intestinal wall. The disease can also be transmitted by subcutaneous inoculation of an emulsion of the intestinal mucous membrane which has been passed through a Berkefeld N filter. Rodier(1) infected ten of eleven fowls which he inoculated with a Berkefeld filtrate of the intestinal mucous membrane. All developed typical symptoms of the disease with the exception of one. In another experiment thirteen fowls were inoculated with a Berkefeld filtrate of an emulsion prepared with the contents of the crop. Ten of these fowls developed typical symptoms of the disease, while three appeared to be resistant. The virus is apparently present in the blood stream of infected fowls only in the early stages of the disease. Bacteria isolated from the fæces of infected animals appear to be innocuous. Injection of liver, kidney, and lung tissues from infected fowls into healthy fowls produces the disease in some instances. Bile in one instance has proved infective. Brain emulsions prepared from brain tissue of birds that have died of the disease have in all instances proved negative.

No attempt to cultivate the virus of this disease has as yet been made. From these preliminary experiments there appears to be strong evidence that this disease is caused by a filterable virus and represents a disease of fowls heretofore unknown. Since January the spread of the disease has been effectively checked by rigid quarantine measures. The causative filterable virus has been passed serially through a large number of fowls and a more complete study of the virus, the disease, and its pathology is contemplated.

What appears to be a similar if not identical disease was reported by Doyle(2) in England in June, 1927. In any event if these two diseases in fowls should prove to be identical they were observed independently by these two investigators in different parts of the world.

BIBLIOGRAPHY

1. Rodier, Proc. Exp. Biol. and Med. 25 (1928) 781.
2. Doyle, Journ. Comp. Path. and Therap. 40 (1927) 144.

ILLUSTRATIONS

PLATE 32

FIG. 1. Chicken pox. Epithelial proliferations on the comb, on the wattles, and near the corners of the mouth. (After Hutyra and Marek.)

2. Fowl diphtheria. Heavy, dry, caseous deposits in the left corner of the beak; small deposits on the tongue. (After Hutyra and Marek.)

3. *Chlamydozoon* (*Strongyloplasma*) *avium.* Smear from buccal mucous membrane of a pigeon having fowl diphtheria (Giemsa staining). After Hutyra and Marek.)

PLATE 33

FIG. 1. Fowl paralysis. Posture assumed by chicken in one stage of paralysis. (After Pappenheimer, Dunn, and Cone.)

2. Fowl paralysis. Posture assumed by chicken in one stage of paralysis. (After Pappenheimer, Dunn, and Cone.)

3. Fowl paralysis. Dissection of spinal cord and brachial plexus in a typical case of fowl paralysis. On the right side there is massive nodular thickening of the nerve trunk extending into the corresponding segment of the cord. (After Pappenheimer, Dunn, and Cone.)

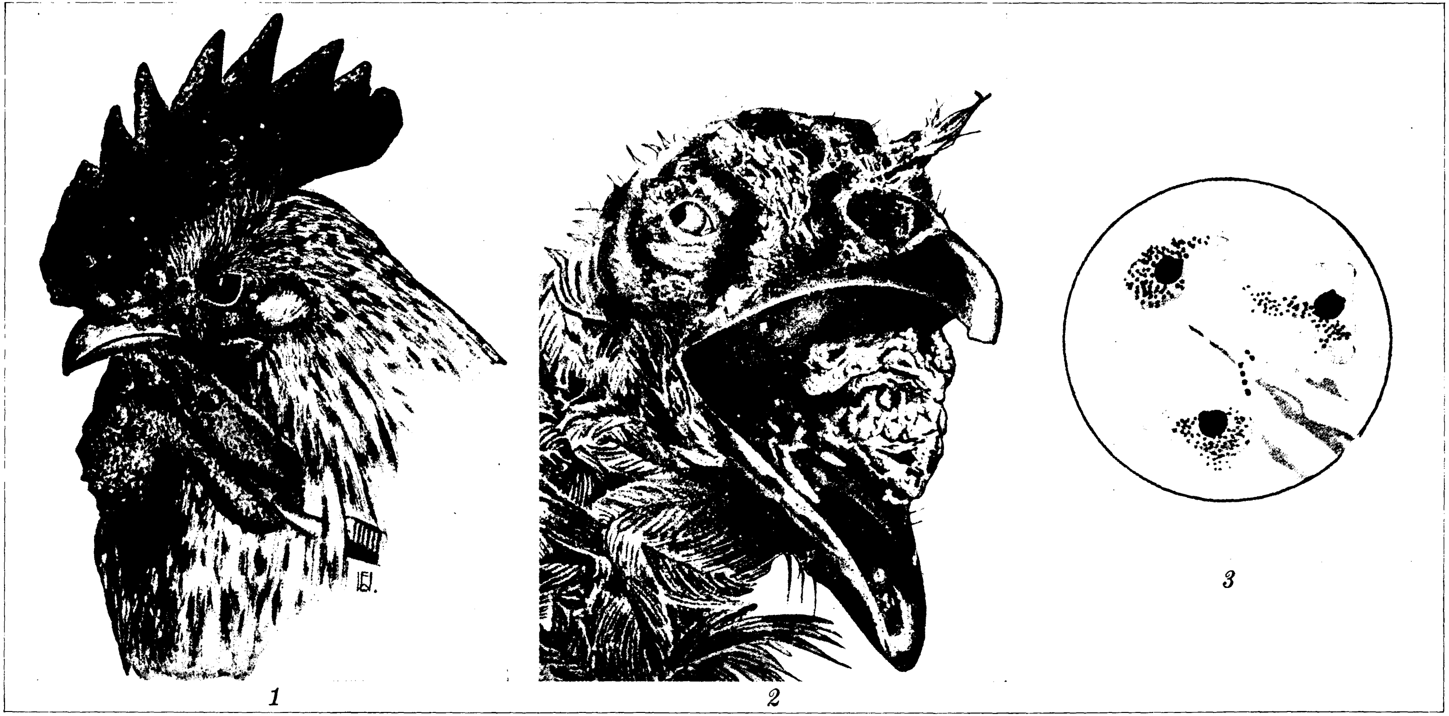

Fig. 1. Epithelial proliferations in chicken pox. 2. Fowl diphtheria; heavy, dry, caseous deposits. 3. Chlamydozoon (Strongyloplasma) avium, smear from buccal mucous membrane of a pigeon.

PLATE 32.

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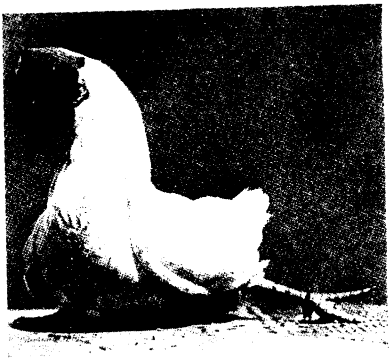

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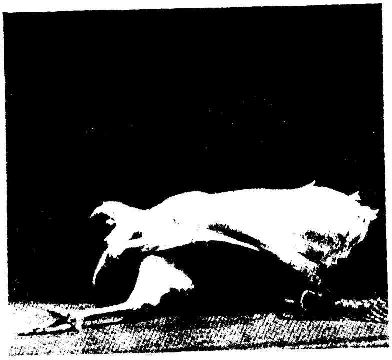

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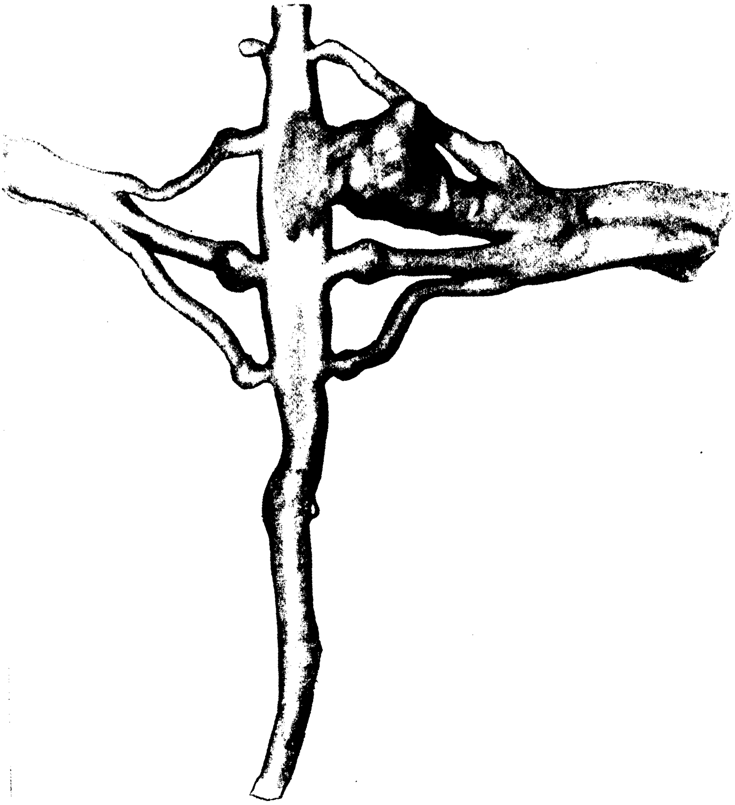

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Figs. 1 and 2. Postures assumed by chickens in fowl paralysis. 3. Dissection of spinal cord and brachial plexus in a typical case of fowl paralysis. On the right side there is massive nodular thickening of the nerve trunk extending into the corresponding segment of the cord.

PLATE 33. FOWL PARALYSIS.

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CHAPTER XII

FILTERABLE VIRUS DISEASES OF INSECTS

SACBROOD DISEASE OF BEES

Definition.—Sacbrood is an insidious infectious disease of the brood of bees. According to White(1) the disease does not attack adult bees but infects larvæ. It is caused by a filterable virus.

History.—For our knowledge of the true nature and etiology of this disease we are indebted to White. In this author's historical account of the disease it appears that as early as 1857 Langstroth(2) recognized two types of "foulbrood," the dry and the moist. White believes that the dry foulbrood described during the last century might easily have been sacbrood. Likewise a brood disease described by Doolittle(3) in 1881 as similar, yet different from foulbrood, may have been sacbrood disease. In White's opinion the disease described by Jones(4) in 1883, which was somewhat different from the genuine foulbrood, was also probably sacbrood disease. Later reports of brood disease by Cook(5) (1904) in California, Burri(6) (1906) in Switzerland, and Kursteiner(7) (1910) of Switzerland, are all considered by White to have been instances of sacbrood disease. Up until this time no name had been given to the disease, though in different parts of the world dead brood resembling foulbrood, yet differing from this disease, had been observed. In 1913, after a careful study of this disease, White ascribed to it the name of sacbrood because of the sacklike appearance of the dead larvæ. Since then it has been known by that name.

Distribution.—The disease has been reported from various parts of Germany, Switzerland, Australia, Denmark, England, and the United States. It is probable, according to White, that many reports of so-called "pickled brood" of bees, a disease originally described by Howard(8) in Texas as being caused by a fungus, *Aspergillus pollini*, were in reality instances of sacbrood disease. In White's experience "pickled brood" disease of bees has never been met with and probably does not exist.

The virus of sacbrood disease.—In 1908 Dadant(9) expressed the belief that sacbrood disease is infectious. Subsequent work by White has proved that this is so. In 1913 this author demonstrated that the disease could be produced at will by feeding the crushed tissues of larvæ dead of sacbrood, suspended in sugar sirup, to healthy colonies. No demonstrable microörganisms could be found either by microscopic or cultural methods in the crushed larval tissue, yet Berkefeld filtrates of this tissue when added to sugar sirup and fed to health colonies produced the disease. Filtrates prepared with the Pasteur-Chamberland filter were also found to contain the virus and produced the disease in healthy colonies. From these experiments of White it may be concluded that the cause of sacbrood disease of bees is a filterable virus. According to this author the virus contained in one dead larva is sufficient to produce the typical disease in at least 3,000 healthy larvæ. It was further demonstrated that the virus of sacbrood when suspended in water and heated to 59° C. was destroyed in ten minutes. The virus when suspended in honey and heated to 70° C. was destroyed within ten minutes. The virus of sacbrood resists drying at room temperature for about three weeks. Direct sunlight destroys it in from four to seven hours. The virus remains viable when suspended in honey and protected from light for nearly one month. Fermentative processes in sugar solutions containing the virus of sacbrood destroys it within a few days. The virus is not destroyed within three weeks by a 2 per cent solution of carbolic acid. The virus of sacbrood has not been cultivated, and little is known of its nature.

Incubation period of sacbrood.—In the experience of White the first symptoms of the disease appear after inoculation of healthy colonies with infected material, in five to six days. This may be regarded as the period of incubation for the disease.

Symptoms.—As White points out—

> in the matter of diseases in practical apiculture the beekeeper is interested primarily in the colony as a whole, and not in individual bees. Therefore, in describing the symptoms of a bee disease, the colony as a whole should be considered as the unit for description, and not the individual bee.

In general the first symptom of sacbrood is the presence of dead brood. Irregularity in the appearance of the brood nest may be noted. If many members of the colony are initially infected, a loss in strength may be observed. Brood dying of the disease are usually found in capped cells before the pupal stage is reached.

White states:

> It is rare to find a pupa dead of sacbrood. The larvae that die are found lying extended lengthwise with the dorsal side on the floor of the cell. They may be found in capped cells or in cells which have been uncapped, as bees often remove the caps from cells containing dead larvae, . . . sometimes a cap has a hole through it which suggests by its position and uniform circumference that it has never been completed. Through such an opening or through one of the larger punctures the dead larva may be seen within the cell. The larva recently dead of sacbrood is slightly yellow. The color in a few days changes to brown. The shade deepens as the process of decay continues, until it appears in some instances almost black. . . . If the dead larva is not removed, its surface through evaporation of its watery content, becomes wrinkled, distorting its form. Further drying results in the formation of the "scale." This scale is not adherent to the cell wall.
>
> No signs in a larva dying of sacbrood have yet been discovered by which the exact time of death may be determined. As the larvae in this disease usually die during the time when they are motionless, lack of movement cannot be used as an early sign of death. In this description it is assumed that the larva is dead if it shows a change in color from bluish-white to yellowish or indications of a change from the normal turgidity to a condition of flaccidity.
>
> The appearance of a larva dead of sacbrood varies from day to day, changing gradually from that of a living healthy larva to that of the dried residue—the scale. A description that would be correct for a dead larva on one day, therefore, may and probably would be incorrect for the same larva on the following day. Moreover, all larvae dead of the disease do not undergo the same change in appearance, causing another considerable range in variation.

White has divided the changes in the larva infected with sacbrood into five arbitrary stages, and the reader is referred to his original work for a detailed account of these changes. Suffice it to say that the virus of sacbrood remains viable through the second stage but thereafter is found to be innocuous when healthy colonies are fed with such material.

Immunity in sacbrood disease of bees.—Under the heading of predisposing causes White has touched upon this subject. It appears from this author's work that adult bees are not directly susceptible to the disease. Pupæ are rarely affected. The larvæ are most susceptible and usually become infected about two days before the pupal stage. Both worker and drone larvæ are susceptible. White states that queen larvæ apparently are also susceptible, although this point has not yet been completely demonstrated. No racial immunity has been established. There are probable differences in degree of susceptibility. White concludes that blacks are more susceptible than strains having Italian blood in them. Climate is not known to have any bearing upon the appearance of the disease, and the experimental disease has been produced by White without relation to season. Quality or quantity of food is said to have little if any bearing upon the appearance of the disease. Colonies tend to recover from the disease without treatment.

Pathology.—The anatomical changes produced by this disease in the larva are quite characteristic. The gross appearance of the larvæ after death has been described. According to White sections through one of these larvæ shows that the bulk of the body is composed of fat tissue. The fat cells are irregular in outline with an irregular-shaped nucleus. Black-staining spherical bodies are to be found within the cells. This gives the contents of the larva a granular appearance. During decay there is a considerable intercuticular space which is filled with a coagulable fluid. The body wall of the larva has a cuticula, a hypodermis, and a basement membrane. Hypodermal cells may be found in the mass content of the larval remains. Other cellular elements such as œnocytes and unidentified cells larger than fat cells are also found. The œnocytes do not contain the dark-staining bodies such as are found in the fat cells. The foregoing cellular composition of the larval remains taken together with cells coming from various organs make up a composite affair. The granular appearance of the mass is due chiefly to the fat cells suspended in liquid. The nature of the dark-staining bodies found in these cells is not definitely known. Possibly they may be related to other inclusion bodies that are characteristic of several filterable virus infections.

Control measures.—The modes of transmission of this disease within a colony and from colony to colony is little understood. It is theoretically possible that the virus may be transmitted from contaminated flowers or by water supply or even by visiting bees, but these possibilities have not been determined. According to White, robbing may possibly be a means of transmission since robbing occurs when the colony is weakened. No suitable or efficient methods for the control of this disease have been suggested. Entire colonies may be wiped out with sacbrood, but this is the exception; the tendency is for the colony to recover. In this respect the prognosis is good.

BIBLIOGRAPHY

1. White, 11th Ann. Rpt. Comr. Agr. N. Y. 1903 (1904) 103–114; U. S. Dept. Agr. Bur. Ent. Bull. 75 pt. 4 (1908) 33–42; Circ. 169 (1913) 5 pp; U. S. Dept. Agr. Bull. 92 (1914) 4; Bull. 431 (1917) 54 pp. (lit.).

2. LANGSTROTH, A Practical Treatise on the Hive and Honey-Bee, 2d ed. (1857) 275, illus.
3. DOOLITTLE, In Gleenings in Bee Culture **9** (1881) 118–119.
4. JONES, Am. Apiculturist **1** (1883) 79–80.
5. COOK, The Bee-Keeper's Guide or Manual of the Apiary, 18th ed. Chicago (1904) 543 pp., 295 figs.
6. BURRI, Bakteriologische Unterschungen über die Faulbrut und Sauerbrut der Bienen Aaran, Switzerland (1906) 40 pp.
7. KURSTEINER, Schweizerische Bienen-Zeitung, Yahrg. **33** (1910) 187–89.
8. HOWARD, Am. Bee Journ. **36** (1896) 577; ABC of Bee Culture (1903) 157–158.
9. DADANT, Diseases of Bees. Langstroth on the Hive and Honey-Bee. Hamilton, Ill. (1908) 487.

WILT OF THE GYPSY-MOTH AND THE EUROPEAN NUN-MOTH CATERPILLAR

POLYHEDRAL DISEASE OF CATERPILLARS

Definition.—The wilt disease of gypsy moth is an infectious disease of caterpillars that is characterized by a disintegration of the tissues of the caterpillar in which are found myriads of polyhedral bodies of various sizes. The disease is caused by a filterable virus.

History and distribution.—According to Glaser(1) the gypsy moth was first brought to the United States, Medford, Massachusetts, in 1869. It is said not to have become serious until 1889. This author has found no account of the wilt disease affecting the caterpillar prior to 1900, and there is still much speculation as to how and when this disease was introduced into the United States. Glaser believes that the wilt disease of the European nun moth, Wipfelkrankheit, may be identical with the wilt disease of the gypsy moth and that it may have been introduced from Europe on trees and shrubbery or other material. Since caterpillars which die of the disease are found disintegrated and dried on trees this explanation seems possible. Glaser states that "this seems very likely in the light of recent investigations by Escherich and Miyajima,(2) and Prowazek(3) on the long resistance to drying of the virus of Wipfelkrankheit and Gelbsucht." This idea is further supported by the fact that in caterpillars dead of both the wilt disease of the European nun moth and the wilt disease of the gypsy moth, the characteristic polygonal or polyhedral bodies are found in the tissues.

In 1905 the State of Massachusetts and the Federal Bureau of Entomology imported parasites and natural enemies of the gypsy moth from Europe and Japan. The wilt disease may have been introduced with these parasites. The first report on the wilt disease of the gypsy moth was published in 1907, by Howard and Fiske.(4) The author also points out that the wilt disease of the tent caterpillar and that of the gypsy moth may possibly be identical and that the latter insect may have become susceptible to the disease of the former. However, there is no experimental evidence of the identity of these two diseases.

In 1915 Glaser reported that Maine, New Hampshire, Massachusetts, and Rhode Island were all infested with the gypsy moth, and the wilt disease was known to be present in these areas. The gypsy-moth caterpillars mature in July, and after they have exfoliated the trees, they are exposed to the direct rays of the sun. Also there is naturally a lack of food. These factors contribute to the spread of the disease by lowering the resistance of the caterpillars. It is believed that the sunlight can convert the chronic form of wilt into the acute form.

The virus of wilt disease of the gypsy-moth caterpillar.—According to Glaser's experiments caterpillars fed with Berkefeld filtrates of material from caterpillars dead of wilt disease develop the affection. The filtrates contain neither bacteria nor polyhedral bodies, yet the typical disease is produced and the infected caterpillar contains large numbers of the characteristic polyhedral bodies. These bodies have been found in both the wilt disease of the European nun moth and in the wilt of the gypsy-moth caterpillar. The average size of these bodies is from 1 to 6 microns. The limits of variation are from 0.5 to 15 microns. They are shaped like a polyhedron with rounded angles and are never spherical. An actual geometric outline has not been observed by Glaser, as in the case with the silkworm polyhedra that are almost perfect octahedra. These bodies are highly refractive, and the center is denser than the periphery. Onionlike concentric layers are sometimes observed. On pressure in a glass-slide preparation these bodies crack into a number of pieces. This sometimes occurs spontaneously without pressure. Pigment granules that resemble bacterial forms are found in smear preparations. True bacteria are rarely found. In fresh preparations very minute dancing granules that may come from the polyhedral bodies are to be seen. Within pathologic nuclei very minute dancing granules can be seen. Glaser suggests that these granules may be particles of degenerated chromatic or achromatic substance, but he is inclined to the view that they represent extremely minute microörganisms. These granules may represent the vegetative stage of the polyhedral bodies or the polyhedra may be a secretion of a minute organism contained within. These small dancing granules, however, are found in the Berkefeld filtrates, while the polyhedra and other organized structures are removed. Glaser states:

> As long as there is no evidence, however, that the polyhedral bodies are directly related to the filterable virus or to the little granules, the view that they are reaction products appeals more strongly. The virus invades the nuclei of the hypodermal, fat, tracheal matrix, and blood cells, and the polyhedral bodies arise, perhaps, as by-products of nuclear digestion and disintegration.

The polyhedral bodies are found within the nuclei of the hypodermal, fat, and blood cells, and also within the nuclei of the tracheal matrix cells. Stained sections demostrate that they originate here, but Glaser was unable to find them present in the nuclei of the muscles, Malpighian tubes, ganglia, or nerves. Further, such bodies have not been demonstrated within the nuclei of gland cells. While the true significance of the polyhedral bodies is at present little understood, it appears that they may be related to so-called inclusion bodies which have been described in other diseases. The minute dancing granules observed within the nuclei and the Berkefeld filtrate may be etiologically significant. Further study is necessary to decide the significance of these important observations. The polyhedral virus has not been cultivated; it is filterable through Berkefeld N filters but not through the Pasteur-Chamberland filter; it is destroyed by heating to 60° C. for twenty minutes and by dry heat at 70° C. for the same length of time; the virus resists drying at room temperature for two years; it is resistant to glycerin; it is destroyed by 5 per cent carbolic acid within three weeks and is destroyed by alcohol within a few minutes. The available data indicate that the virus of wilt disease is a living agent such as other ultraviruses having similar properties.

Incubation period.—The incubation period in experimentally produced wilt disease of the gypsy moth varies from two to twenty-five days. Temperature appears to bear a very important relation to this variation.

Susceptible insects.—The virus described by Glaser is infectious for the gypsy-moth caterpillar. In the author's opinion it is quite probable that the European nun moth is also affected, indeed may have been first affected by this virus, and through importation from Europe entered the United States. While there is a possibility that the tent caterpillar may also be affected by this virus, there is no experimental proof bearing on the subject and in Glaser's opinion this is unlikely.

Immunity.—Genetic immunity of certain individuals is quite probable, and there is evidence that active immunization with sublethal doses of infectious material is possible. While it is probable (Glaser), there is no evidence that wilt is transmitted from one generation to another.

Pathology.—The histological findings in this disease may be summed up briefly as follows: The pathology of wilt does not vary with the age of the caterpillars; the polyhedral bodies originate in the nuclei of hypodermal, tracheal matrix, fat, and blood cells; minute dancing granules that stain with Giemsa's stain are found within the nuclei of fresh diseased-tissue preparations; the intestinal canal is last to disintegrate as a result of the disease; according to Glaser two types of blood corpuscles exist in diseased caterpillars. The reader is referred to Glaser's original publications for a more-detailed description of these changes.

BIBLIOGRAPHY

1. GLASER and CHAPMAN, Science, n. s. **36** (1912) 219–224; Journ. Econ. Ent. **6** (1913) 479–488.
   GLASER, Journ. Agr. Res. **4** (1915) 121–128 (lit.); Science, n. s. **48** (1918) 301–302.
2. ESCHERICH and MIYAJIMA, Naturw. Ztschr. Forst-u. Landw., Jahrg. **9**, Heft 9 (1911) 381–402.
3. PROWAZEK, Centralbl. f. Bakt., Abt. 1, Orig., Bd. **67**, Heft 4 (1912) 268–284.
4. HOWARD and FISKE, Bull. U. S. Dept. Agr. Bur. Ent. **91** (1911).

JAUNDICE OF SILKWORMS

GELBSUCHT; GRASSERIE

Jaundice of silkworms was first described by Nysten(1) in 1808. The disease is characterized by a turbid yellowish blood in the yellow and green races, and a whitish turbidity in the blood of the white races. Jaundice of silkworms is present nearly everywhere that worms are bred and has been prevalent in Japan and parts of Europe, particularly, for many years. The Japanese speak of this disease of the silkworm as "Nobyo," and the diseased worms are designated "Nosan" or "Umiko." Dandolo(2) in 1925 characterized the vaches, gras, or yellows as follows: First the head of the worm swells, then the skin is drawn tight over the rings and shines as with varnish. The rings then swell. This is followed by the circumference of the aperture of the stigmates becoming light or deep yellow in color. Finally "the worm voids a yellow liquid which may be seen on the leaves."

Since its first description this disease has attracted the attention of many investigators, all of whom have added their observations of the malady to the literature. A review of the literature is given by Sasaki(3) in a description of his work on this disease published in 1909–1911 and need not be recounted here. Since the earliest descriptions of the disease there has been quite general agreement regarding its manifestations, although its etiology has long been a moot question.

Sasaki originally began his work on the jaundice of silkworms in conjunction with Bolle.(4) In 1894 Bolle pointed out that the polyhedral bodies found in the blood of silkworms dead of jaundice are in reality a species of Coccidæ and in his opinion the real cause of jaundice in silkworms. Later, in 1895, Sasaki described polyhedral bodies in the fatty and muscular tissues, peritracheal membrane, and silk glands of the worms and advanced the theory that these bodies were formed within these organs and tissues as the result of insufficient respiration. Later studies on this disease by Sasaki, however, have led to the opinion that the polyhedral bodies are crystalloids and are formed within the organs and tissues as a result of certain diseases and unhealthy conditions under which the worms are bred.

In 1896 Krassilschtschik(5) demonstrated the presence of a micrococcus in the blood of worms having jaundice and concluded that this microbe, *Micrococcus lardarius,* is the specific cause of the disease. Again, in 1898, Bolle stated that he believed that the polyhedral bodies (poledrischen Körnchen) were parasites and the cause of the disease. He further described their reproduction and suggested the name *Microsporidium polyedricum* by which to designate them. Later, in 1903, Bolle showed that these parasites possess infective power for other insects, such as *Bombyx, Autherea,* and *Attacus.*

Miyabara and Yanai,(6) Omori,(7) and Tamura(8) all agreed with Bolle that the disease was caused by a parasitic sporozoan. In 1907 Prowazek(9) described *Chlamydozoa bombycis* as the cause of jaundice of silkworms. During the same year Conte and Levrat(10) concluded as a result of their studies that the polyhedral bodies found in this disease are the products of degeneration of adipose and other tissues. The precise status of the polyhedral bodies then is not settled. By some they are regarded as the spores of *Microsporidium polyedricum,* by others as the reaction product of the cell to the presence of *Chlamydozoa bombycis,* and by others as the degeneration products of cells.

Sasaki regards the appearance of polyhedral bodies as a symptom of jaundice in silkworms rather than a cause. He is inclined to the belief that the causes of jaundice in silkworms are multiple since he has been able to induce the disease, with the appearance of polyhedral bodies, by decreasing respiration, by inoculating healthy worms with various strains of streptococci obtained from the blood of silkworms, by feeding healthy worms leaves on which formalin had been placed, and by feeding healthy worms with the leaves of *Cudrania triloba* and *Taraxacum officinale,* plants more or less foreign to silkworms.

The polyhedral bodies found in silkworms or pupæ affected by jaundice are flattened, thin, many-sided plates; but they appear mostly six-sided. These bodies may be crushed by pressure between two glass slides. They frequently become crushed in drying and are broken up by temperatures ranging from 140 to 150° C. These bodies are heavier than water and fall to the bottom in water suspensions. They vary in size from 0.0066 to 0.008 millimeter in diameter. They stain with carbolfuchsin, picric acid, and other dyes. They are not affected by concentrated sulphuric or nitric acid, caustic potash, ether, or chloroform. Sasaki found that the polyhedral bodies in diseased worms that had been kept in alcohol for three years retained their normal shape. He regards these bodies as crystalloids.

The polyhedral bodies are always produced within the nucleus of the cell and not in the cytoplasm. Sasaki states:

> In the nucleus of some cells, there can be seen certain dots or granules having more or less angular or polyhedral shape. In other cells, the nucleus contains more or less larger dots or granules having polyhedral shape. These granules grow larger and larger within the nucleus, until the latter is nearly filled with them. Furthermore, the nucleus grows larger until it nearly fills the cell. In many cases, as a result of the abnormal growth of the nucleus, the membrane of the cell in which it is embedded ruptures, and the nucleus containing the granules becomes free and is thrown into the blood; or at the same time with the rupture of the cell membrane, the nuclear membrane breaks up too, and the granules contained in the nucleus are thrown out into the blood.

Sasaki believes that the sacs containing the polyhedral bodies which are seen in the blood represent nuclei rather than cysts of *Microsporidium polyedricum* described by Bolle. The same effect may be given by blood cells containing polyhedral bodies.

The symptoms of jaundice in silkworms appear usually in the fifth stage. Frequently the diseased worms appear at each molt. Dead worms or pupæ, imprisoned within their coccoons, often contain polyhedral bodies, and parasitic maggots that come out of the silkworms or pupæ also contain the crystalloids within their bodies.

The etiology of jaundice in silkworms remains obscure. Sasaki has been able to induce symptoms of the disease by various methods, but a specific etiologic agent has not been discovered. Many investigators think this disease is caused by a member of the filterable virus group as evidenced by the inclusion bodies described by Prowazek. Careful filtration experiments will be necessary to substantiate this theory.

Other filterable virus diseases of insects.—According to Paillot(11) polyhedral disease of the caterpillar of the black arches moth, *Hymantria monacha,* is also caused by a filterable virus as evidenced by the polyhedral bodies present in this affection which are similar to those already described. Furthermore, there is a form of grasserie which affects the caterpillar of the large, white, cabbage butterfly and also a nuclear disease affecting this caterpillar associated with the presence of refringent rings in the cytoplasm of blood cells and fat cells. Very little is known concerning these diseases.

BIBLIOGRAPHY

1. NYSTEN, Recherches sur les Maladies de Vers á Soie (1808).
2. DANDOLO, The Art of Rearing Silkworms (English translation) (1825) 272.
3. SASAKI, Journ. Coll. of Agr. Tokyo (1909–11) 105–159 (lit.).
4. BOLLE, Die Krankheiten des Maulbeerseidenspinners (1875); Kurz Anleitung zur rationellen Aufzucht der Seidenraupe (1881); Der Seidenbau in Japan (1898) 105; Bericht über die Thätigheit der K. K. Landw.-chemischen Versuchsstation (1903) 8.
5. KRASSILSCHTSCHIK, Compt. Rend. (1896) 425–429.
6. MIYABARA and YANAI, Special Report of Fukushima Sericultural School (1903) (Japanese).
7. OMORI, Report of Dai Nippon Sanshi Kwaiho (1905) Nos. 42, 43, 44.
8. TAMURA, Report of Dai Nippon Sanshi Kwaiho (1907) No. 178.
9. PROWAZEK, Archiv. f. Protistenkunde **10** (1907) 2 and 3, 363.
10. CONTE and LEVRAT, Les Maladies du Ver a soie. La Grasserie. Laboratoire d'Études de la Soie **13** (1906–07) 57.
11. PAILLOT, Anns. l'Inst. Pasteur **40** (1926) 341 (lit.).

ILLUSTRATIONS

PLATE 34

FIG. 1. Marked sacbrood infection. (After White.)

2. Heavy sacbrood infection showing a number of different stages of decay of larvæ. Eggs, young larvæ in different stages of development, and diseased larvæ in same area. (After White.)

PLATE 35

Comparison of a healthy larva and the remains of larvæ dead of sacbrood; *a*, cap of a healthy larva; *b*, *c*, *d*, *e*, and *f*, caps over larvæ in first, second, third, fourth, and fifth stages of decay; *g*, a healthy larva; *h*, *i*, *j*, *k*, and *l*, end views of five stages of decay; *m*, healthy larva; *n*, *o*, *p*, *q*, and *r*, five stages of decay from end view; *s* and *y*, healthy larvæ; *t*, *u*, *v*, *w*, and *z*, stages of decay of larvæ removed from cells; *ww*, larva recently dead of sacbrood; *x*, scale; *xx*, larval remains; *yy*, almost a pupa; *zz*, pupa dead of sacbrood. (After White.)

PLATE 36

FIG. 1. Healthy larva and cell viewed from above and at an angle. (From White.)

2. Scale, or larval remains, in position in cell cut lengthwise, lateral view. (From White.)

PLATE 37. POLYHEDRA OF GYPSY-MOTH CATERPILLARS. (AFTER GLASER.)

FIG. 1. Silkworm polyhedron, after Prowazek.

2. Two gypsy-moth caterpillar polyhedra adhering to each other.

FIGS. 3 to 10. Polyhedra of gypsy-moth caterpillar cracking to pieces.

11 to 18. Urate crystals of a gypsy-moth caterpillar.

FIG. 19. Polyhedron of gypsy-moth caterpillar stained, showing a dark central mass.

20. Polyhedron of a gypsy-moth caterpillar stained, showing refractive granules.

21. Chromatic lump in middle of pathological nucleus of a gypsy-moth caterpillar. × 950.

22. Iron hæmatoxylin, showing stained polyhedra of gypsy-moth caterpillar in a nucleus. × 950.

23. Giemsa's stain, showing unstained polyhedra of a gypsy-moth caterpillar in a nucleus and little granules. × 950.

24. Fully formed polyhedra of a gypsy-moth caterpillar in a nucleus. × 950.

25. Nuclear membrane rupturing and allowing polyhedra of a gypsy-moth caterpillar to escape. × 950.

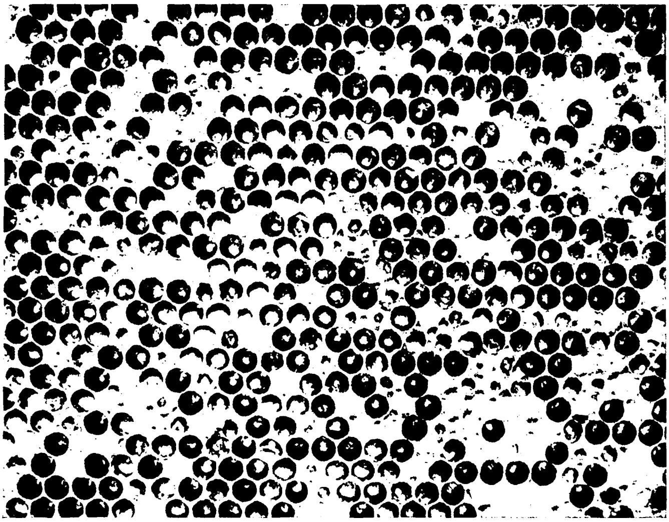
PLATE 38. WILT OF GYPSY-MOTH CATERPILLARS. (AFTER GLASER.)

FIGS. 1 and 2. Normal blood corpuscles of the gypsy-moth caterpillar.

3 and 4. "Mulberry" corpuscles of the gypsy-moth caterpillar.

FIG. 5. "Mulberry" corpuscle of the gypsy-moth caterpillar, showing nucleus (crushed).

6. Blood corpuscle of the gypsy-moth caterpillar, showing nucleus filled with polyhedra.



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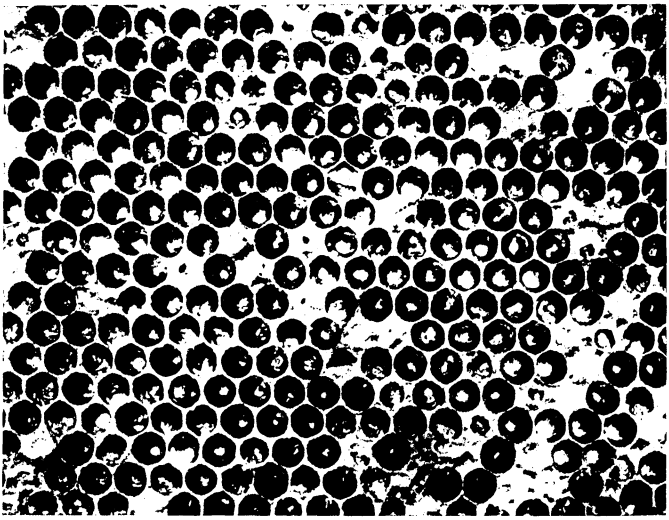

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Fig. 1. Marked sacbrood infection. 2. Heavy sacbrood infection showing a number of different stages of decay of larvæ.

PLATE 34.

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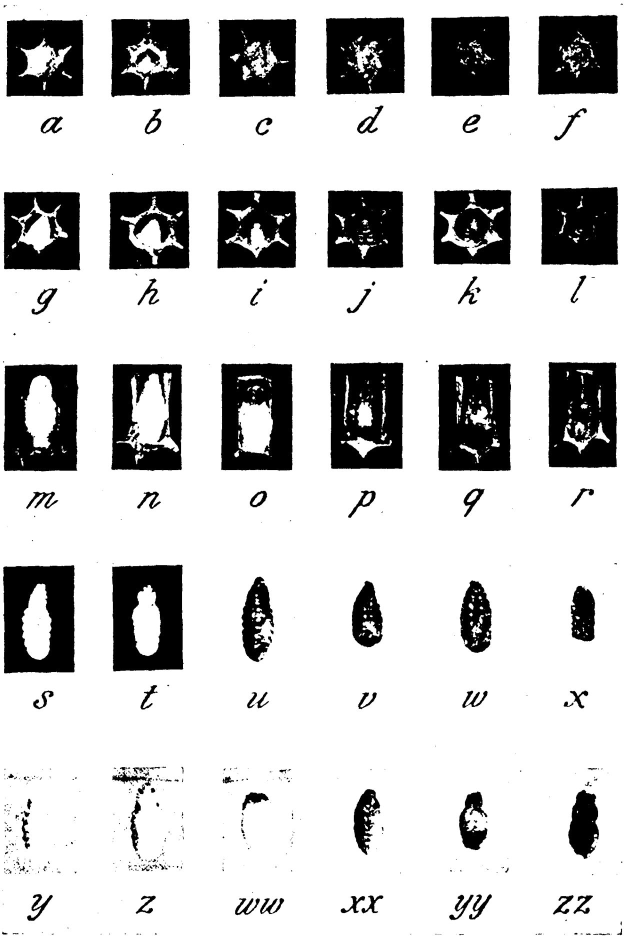

PLATE 35. COMPARISON OF A HEALTHY LARVA AND THE REMAINS OF LARVÆ DEAD OF SACBROOD.

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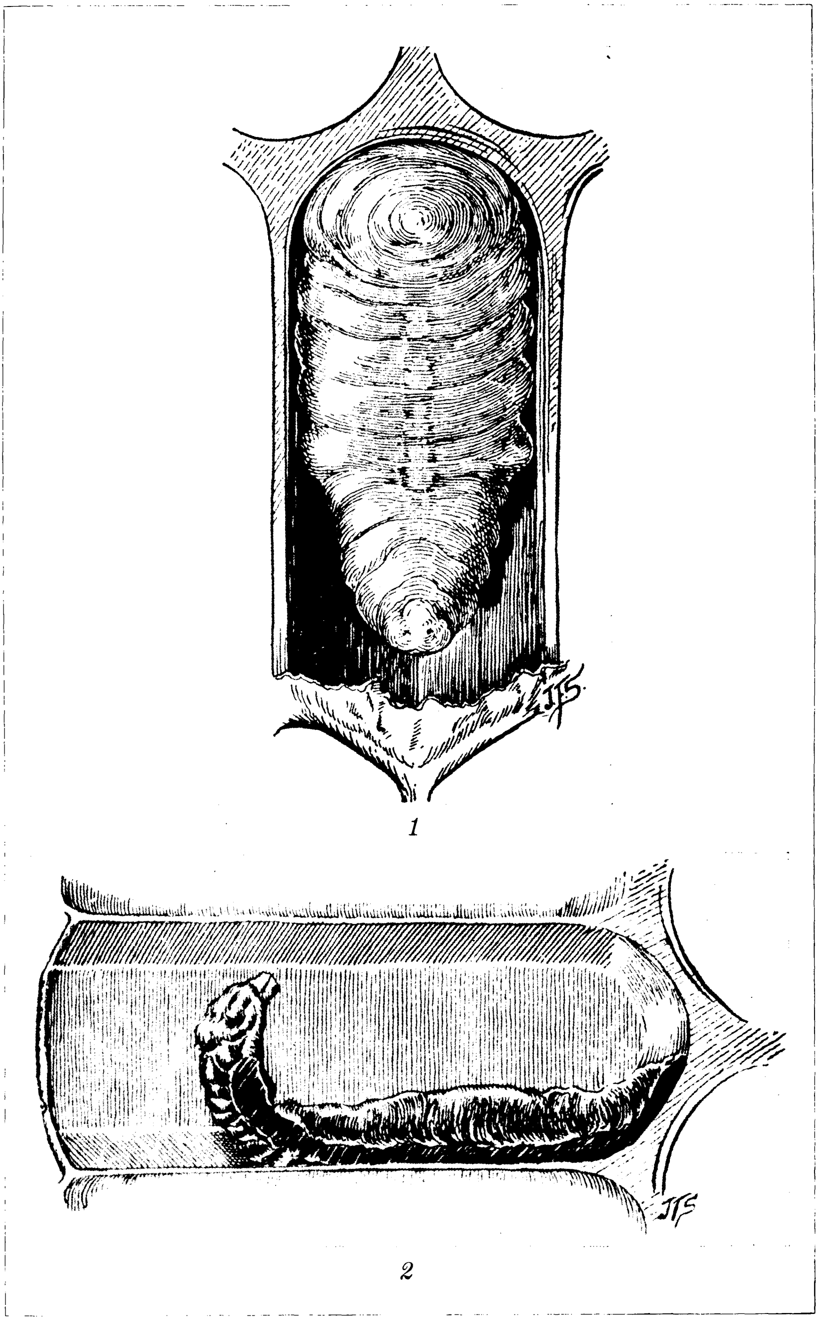

Fig. 1. Healthy larva and cell from above and at an angle. 2. Scale, or larval remains, lateral view.

PLATE 36. SACBROOD OF BEES.

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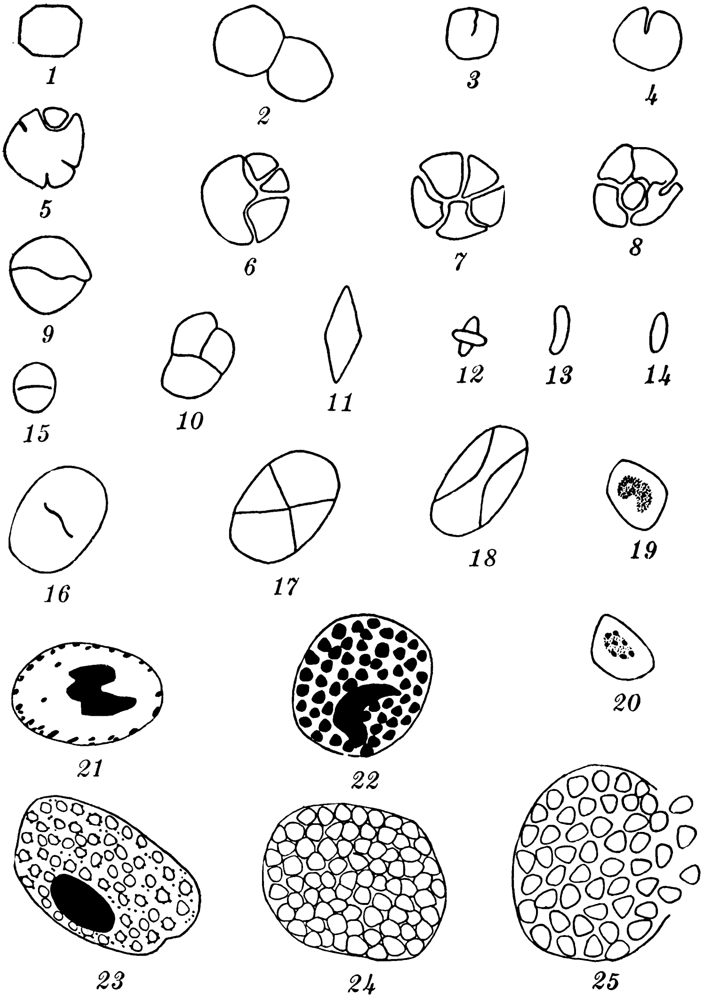

PLATE 37. POLYHEDRA OF GYPSY-MOTH CATERPILLARS.

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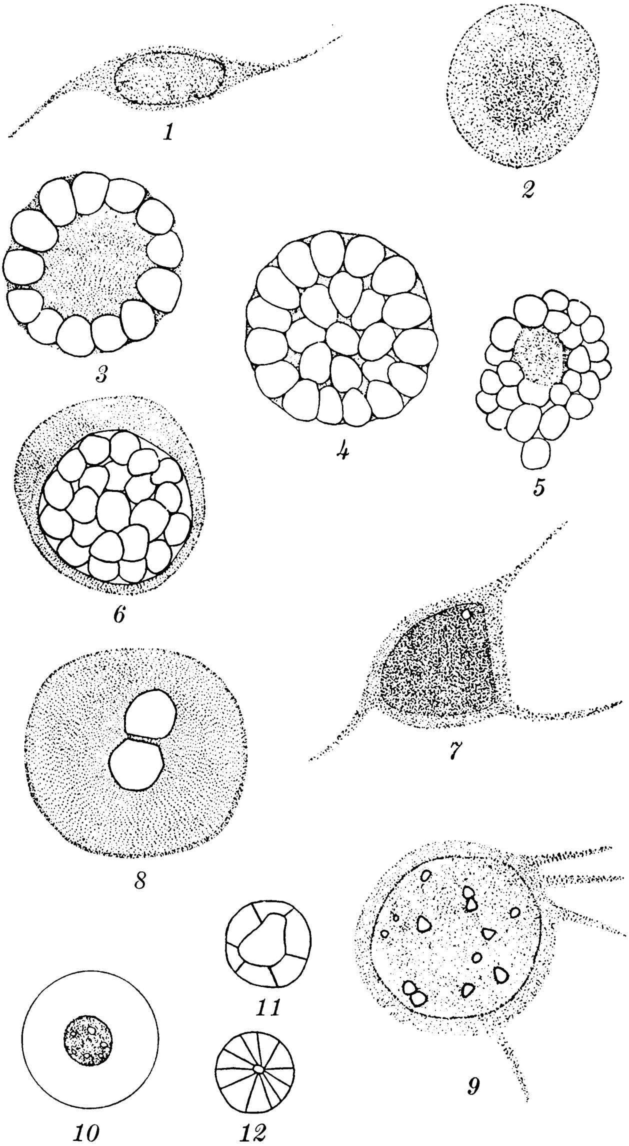

PLATE 38. WILT OF GYPSY-MOTH CATERPILLARS.

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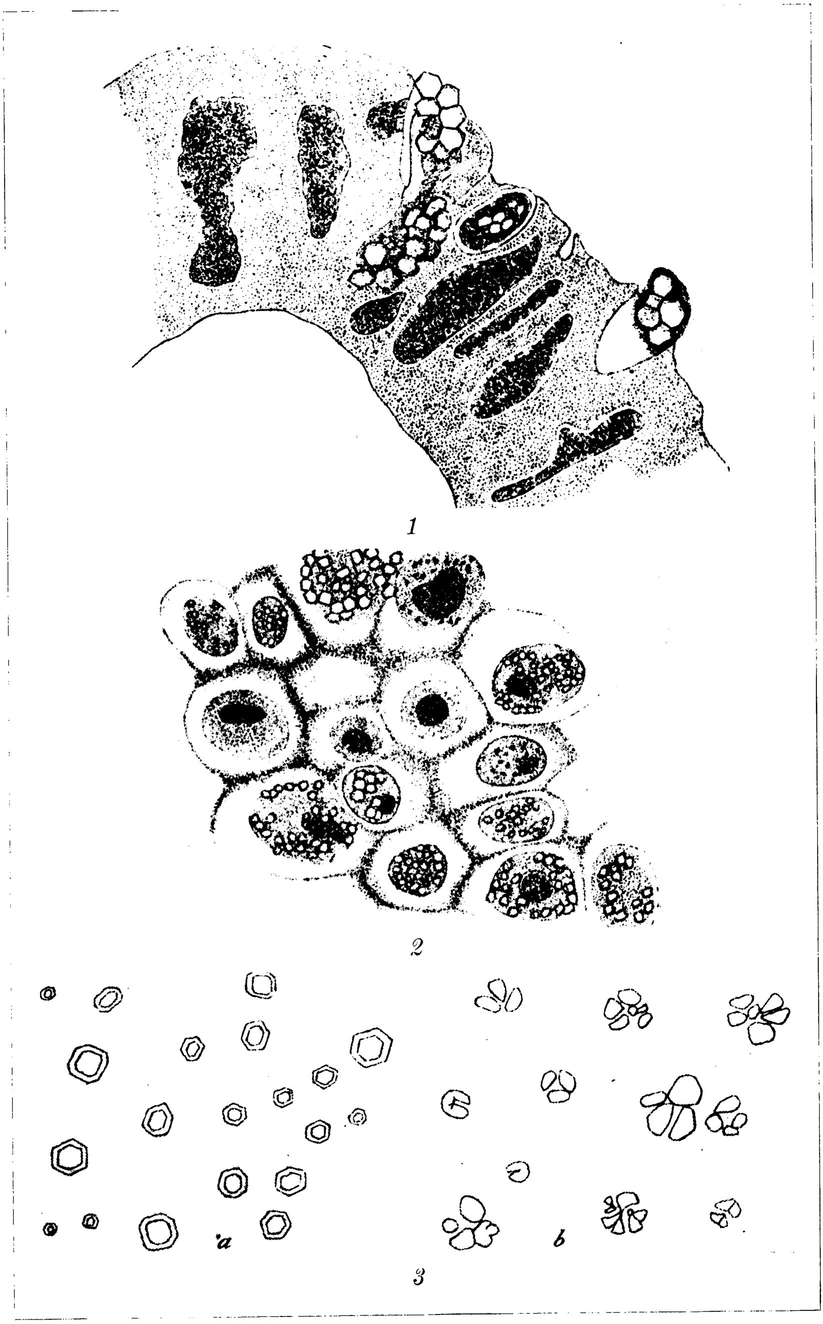

Fig. 1. Diseased silk gland from jaundice of silkworm. 2. Diseased fatty tissue, showing various bodies. 3. Polyhedral bodies; b, deformed polyhedral bodies.

PLATE 39.

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FIGS. 7 to 9. Blood corpuscles of the gypsy-moth caterpillar with phagocytized polyhedra.

FIG. 10. Cytoplasmic-free pathologic blood corpuscles of the gypsy-moth caterpillar.

FIGS. 11 and 12. Crystals found in gypsy-moth caterpillars that died of the "other cause."

PLATE 39

FIG. 1. Section of the diseased silk gland from jaundice of silkworm. (After Sasaki.)

2. Section of the diseased fatty tissue, showing various stages of polyhedral bodies. (After Sasaki.)

3. *a*, Polyhedral bodies; *b*, deformed polyhedral bodies. (After Sasaki.)

CHAPTER XIII

FILTERABLE VIRUS DISEASES OF FISHES

Very little is known regarding the filterable virus diseases of fishes. Practically no experimental work has been done on these diseases further than to recognize and describe some of the various diseases of fishes and to ascertain methods for their control or eradication. Fish culture is very important to the economy of a nation, and in some countries it has been developed upon a very extensive scale. This has made possible the observation and study of many of the affections of this group of animals, and for the most part the diseases of fishes have been found to be caused by bacterial infections or related to other parasites, nutrition, physical phenomena, and environment.

There are three diseases of fishes that have been thought to be caused by filterable viruses. The study of these diseases has been very limited and information concerning them is exceedingly meager. In the light of our present knowledge it would be better perhaps to classify these diseases with the affections of "obscure etiology." However, since these three diseases of fishes have been classified by some investigators with the filterable virus diseases, they will be described briefly in this section.

EPITHELIOMA OF BARBUS

Epithelioma of fishes has been described by Keysselitz(1) as occurring upon the lip of the Moselbarben, or *Barbus fluviatialis* Cuvier. The tumor may be located upon any part of the lip, and very rarely it extends to the skin of the upper jaw. The epithelioma reaches the size of a pea, and usually there is only one tumor although in some cases there may be three or four. On cross section a tumor presents a round or oval base, and is either a flat elevation with a slightly lobulated surface or a conical formation. Its color is the same as the lip, yellowish white, and frequently it is somewhat eroded upon the surface. In a few cases Keysselitz has observed these tumors measuring from 20 to 35 millimeters. As a rule epithelioma of fishes occurs from April to July. It is a general belief among fishermen that tumors occur in the spring but are not observed in the late summer or fall.

Keysselitz has studied the histology of these tumors, and in sections stained with Ehrlich's hæmatoxylin and Breinl's stain for trypanosomes he has demonstrated minute bodies which he believes are Chlamydozoa. He states that these nuclear bodies are comparable to the cellular inclusions of vaccinia, variola, epithelioma of fowls, molluscum contagiosum, trachoma, and rabies, and that they consist mostly of nuclear substance and are the reaction product of the cells to the invasion of minute parasitic microbes—the Chlamydozoa. Due to these observations this disease has been classified with the filterable virus diseases.

The epithelial layer of the skin of the lip of a *Barbus* 35 centimeters in length is about 200 to 400 microns in thickness. There are among the epithelial cells numerous mucous cells which are located in the upper and middle layers. The papillæ are flat. Mitoses are usually absent, but when present they are found only in the superficial layers.

The secretion of the cells when stained with hæmatoxylin does not take the normal blue color but is of a grayish shade. The function of the cell is altered by the disease process. Frequently the mucous cells are found in a stage of disintegration or hyaline degeneration. The leucocytes that are found within the epithelial layers may also show this degeneration. The papillæ are elongated and pointed, reaching deep into the epithelioma.

The changes found within the nuclei of the cells indicate that the process is progressive. In the beginning there are one, two, three, or four round or oval vacuoles within the nucleus. Ultimately the nuclei lose all of their chromatin with the exception of the peripheral chromatin membrane and frequently small islets of chromatin within the nuclear body. Almost regularly there are found one or two, and in some cases, three or four dark-staining, round, oval, or irregular bodies within the changed nucleus. These may show achromatic spots. These bodies may be nucleoli which have lost their tinctorial properties. The intranuclear inclusions begin as small bodies about one micron in size and are surrounded by a transparent halo. These bodies can easily be differentiated from the nucleoli. With hæmatoxylin these bodies take a more intensive blue than the nucleoli. Keysselitz believes that these bodies later divide and grow larger. He states that he has seen as many as eight of these formations in one cell. These bodies he believes represent foreign parasitic elements, the immediate reaction product of which is the capsule consisting of plastin. He believes that these bodies are similar to those found in carp pox.

The theory of a filterable virus origin of epithelioma of fishes is based purely upon histologic evidence. The disease has not been produced experimentally in fishes with filtrates or even with unfiltered material taken from diseased fishes and administered to healthy fishes in an effort to induce the affection. For the present we must conclude that the filterable virus theory is only to be permitted upon the grounds of analogy because certain intranuclear bodies are found, within the cells of the epithelioma, which are similar to inclusion bodies found in other diseases that are known or thought to be caused by filterable agents.

CARP POX

Carp pox was known in the Middle Ages. It was described by Gessner(2) as early as 1563. Hofer(3) states that the disease became more prevalent during the twenty-year period of 1884 to 1904. This author states that carp pox, sometimes called "mushroom disease," occurs in carp and occasionally in other kinds of fishes. It is found in regions where carp are cultivated in ponds, especially where the water is seldom changed. Hofer observed this disease in carp in many parts of Germany and Austria. He states that pox is the most prevalent disease of fishes.

Symptoms and course of the disease.—Carp pox is essentially a disease of the skin. It occurs on the head, fins, and other parts of the body. It first manifests itself as milky, glassy, cloudy, whitish spots, which are at first discrete but gradually become larger and may become confluent. The entire surface of the fish may be covered. The lesions become elevated, being 1 to 2 millimeters in thickness, smooth on the upper surface, and sometimes lobulated. They have a hard consistency and feel like cartilage. At times the growth contains black pigmented stringy material that divides into fine branches. In some instances the growth may have a reddish tinge. The cells of the epidermis are increased in number, indicating an active process. The thickening of the epidermis produces the cloudy, whitish appearance. The epithelial cells also appear cloudy under the microscope. Between the epithelial cells are found a few leucocytes. When the spots have thickened, blood vessels grow in, and when the tumorlike mass is removed it leaves a bleeding surface. A similar disease has been described by Wierzejski(4) in which there is a secondary hypertrophy of the epithelium. This author describes the spots on the skin as mulberry or cauliflowerlike. Hofer does not believe that this disease is carp pox.

During the normal course of the disease the pox spots scale off when they reach a certain thickness but appear again after a short time. They also appear again after they are scraped off with an instrument. In the aquarium it requires from six to eight weeks for the complete development and shedding or casting off of the pox scales. This process is frequently referred to as desquamation. It is continuous in pox-infected fishes, and gradually lowers their resistance and energy. They become thin and atrophied and may finally die of the disease. On the other hand the disease may be benign and not affect the growth. Very little is known of the nature of the disease except that pox-diseased fishes are found in the spring when the fish are collected and the ponds are cleaned.

The virus of carp pox.—The causative agent of carp pox is not known. Hofer searched for years for bacteria and other parasites in the lesions without result. This author finally concluded that the disease is not primary in the skin but that the skin lesions are symptoms of a disease of the internal organs. In the kidneys, and sometimes in the liver and spleen, Hofer found a parasite which he has designated myxosporid. This parasite exists in the form of irregularly formed amœbalike bodies of various sizes in the parenchyma and tubules of the kidneys. It is identified by its spores, 10 to 16 microns in length by 8 to 11 microns in width. Hofer describes polar capsules 5 to 6 microns in length by 3 microns in width. The capsule possesses two membranes. Yellowish refractive bodies are also found in the organs of the carp, but their structure is indefinite. In these bodies are fine brown or black granules. Their origin and composition are unknown. They occur in the stage where the spores are absent. These parasites (?) are found between and in the cells of the kidney and may cause death of the cells. Hofer states that they may be so numerous that the entire kidney consists of parasites. This author states that in the face of such heavy infestation of the kidney it seems likely that the waste products are deposited in the skin of the fish and that the irritation from such products causes the over-growth of the epidermis. According to this view the skin lesions would appear to be secondary to infection of the internal organs of the fish, particularly the kidney. Hofer believes that these observations will also explain the spread of the disease in ponds since the kidney parasites are eliminated by infected fishes and ingested by healthy fishes in their search for food at the bottom of the pond. Hofer suggests that the parasites are eliminated in the fæces of infected carp, sink to the muddy bottom, and are taken up with the food by normal fishes. The carp digest the membrane of the spore, and the sporozoites are then hatched. He further suspects that the sporozoite penetrates the membrane of the gut and is later deposited in the kidney and other organs.

Loewenthal(5) in 1907 described certain inclusion bodies in carp pox similar to those already described for epithelioma of fishes, and upon these observations various authorities have classified this disease with the filterable virus diseases. The exact nature of these inclusion bodies is not known, but their presence in the lesions of carp pox raises some doubt regarding the theory of Hofer as to the etiology of this disease. It is known that the pox eruption regresses when the carp are placed in fresh running water. The spots disappear but reappear again within a few weeks. Complete healing of a pox-diseased fish is impossible according to Hofer, but there is evidence of healing in both the skin lesions and in the internal organs in some instances. Control of the disease depends upon a number of factors. Sanitation of the bottom of fishponds is essential. This is brought about by draining, exposure to the sun, treatment with lime, etc. When the pond is refilled with water only healthy spawn should be introduced.

We may conclude from the information available concerning this disease that the exact cause of the affection is as yet unknown. There is presumptive evidence that the disease may be of a filterable virus origin as evidenced by the presence of inclusion bodies in the skin lesions. On the other hand Hofer's theory should receive serious consideration if only to rule it out.

LYMPHOCYSTIC DISEASE

Lymphocystic disease of fishes is of theoretical and practical interest because it affects certain important and useful fishes. The disease is communicable and can be transmitted with ease to certain species, such as the bass and the flounder. To Weissenberg(6) we are indebted for a considerable portion of our information regarding the nature of this disease, its pathology and cause. Weissenberg states that there are three periods in the investigation of this disease. In the first period investigators described the giant mononuclear cells which appear and proliferate in the skin of the fish and considered these cells ova. In the second period these cells were considered parasitic protozoans. In the last period investigators clearly recognized that these cells are elements belonging to the fish's body organism, elements that hypertrophied as a consequence of the stimulus of an intracellular virus.

Lymphocystic disease of fishes is characterized by the appearance on the skin of spherical nodules that somewhat resemble pearls. The nodules may occur singly or in groups, and frequently, in the latter case, give a cauliflower appearance and resemble the condyloma acuminatum seen in man. Each nodule is composed of a giant lymphocystic cell covered by connective tissue and epithelial cells. In some instances a group of cells, covered with epithelium, are joined together forming a tumor mass. The size of such tumors varies. In flounders they may be 2 millimeters in diameter, but they are usually smaller in bass. In *Sargus* they have been found as large as 400 microns in diameter, and Johnstone(7) found them to measure 320 microns in *Solea.* These nodules develop most commonly upon the fins, but may be found anywhere on the body. In the bass the lymphocystic cells are transparent, and the tumors appear gray and cloudy. The disease ordinarily develops to its fullest extent in the aquarium in from seven to eight months, although it becomes visible to the naked eye within two and a half to three months. Under experimental conditions it has appeared within nine days. Lymphocystic cells are found in the skin, in the deep connective tissue of muscles, etc., and on the bone plates, and even in the spinal canal between the spinal cord and the bones. Woodcock(8) has described these cells as occurring in the mesentery, intestines, stomach, liver, kidney, and ovary. Wherever they are found they are situated in the connective tissue.

Lymphocystic disease of fishes is essentially chronic in its course. After seven or eight months when the nodules are fully developed they tend to regress or degenerate. At this stage they appear as whitish nodules upon the skin. Complete healing may take place and the lymphocystic cells may be cast off. In this case there remains an area denuded of epithelium. The disease is not serious provided it does not involve any of the vital organs. In the aquarium many affected fishes die due to a mold infection of the tumors. This infection is known as "saprolegnia." The chief effect of the disease upon the fish as a whole is to produce an emaciation. Weissenberg believes that the emaciation must be due to the disease since fishes affected with lymphocystic disease and showing emaciation have been found in the Irish Sea. The disease can be produced at any season of the year under experimental conditions, but there seems to be a certain seasonal irregularity in the natural infection that is little known. In freshly caught fishes one can note many different stages of the disease believed to be due to reinfection. This is also noted under experimental conditions.

TABLE 10.—*Distribution of carp pox.*

Host.	Where found.	Author.
Pleuronectes flesus; flounder *Pleuronectes platessa;* Scholle	Parts of seas around England and mouths of rivers; Irish Sea (Luce Bay, Barrow Canal, River Lune). English Channel (River Ouse), North Sea.	Lowe; McIntosh; Sandemann; Woodcock; Johnstone.
Pleuronectes flesus; flounder	North Ice Sea (Barrent's Sea). East Sea, Hidden Sea, Jasmund Bodden. Mouth of River Elbe.	Awerinzew; Weissenberg; Claussen.
Solea vulgaris	East coast of England	Johnstone.
Macropodovia viridiauratus; large macropod crab.	From German Institute for Culture of fish. In 1910 imported from China.	Zschiesche; Max Koch.
Acerina cernua; bass	Jasmund Bodden	Weissenberg.
Sargus annularis: Geisbrasse	Disease was discovered in an aquarium in Vienna. Material came from zoölogical station in Triest, Adriatic.	Joseph.

Microscopic picture of the lymphocystic cell.—According to Weissenberg a definite diagnosis of lymphocystic disease in fishes cannot be made without a microscopic examination. This author describes the lymphocystic cell as follows:

There is a very definite membrane surrounding the cell. The lymphocystic cell is a foreign cell just as a tumor cell is foreign to normal tissue. The lymphocystic cell leads an independent life. The membrane is jellylike and elastic. It is thickened in old degenerated cells. In bass the membrane is more fully developed. In younger cells the nucleus appears like a bubble filled with liquid. It is lobulated and kidney-shaped, eccentrically placed with the hilus toward the center of the cell. The nucleus shrinks when the cells become 100 to 120 microns in diameter. In this stage a network surrounds the nucleus. In shrinking the surface of the nucleus folds over, and later it appears as if pseudopodia come from its surface. Still later the nucleus appears as multiple strands, with depressions between, floating in the plasma of the cell. This observation led Awerinzew(9) to describe this phenomenon as "plastin branching." With the shrinking of the nucleus there is a diminution of the chromatin material. In the latter stages the chromatin is found only on the inner surface of the cell membrane.

The nucleolus is quite distinct in the lymphocystic cells as they occur in the bass. In old cells it is acidophilic, while the cell membrane is basophilic. The plasma is acidophilic, while the granular protoplasm is basophilic. Within the plasma are seen vacuoles, particularly around the nucleus. Between the vacuoles and around their outer surface is seen a pronounced granulation. Besides granules one also notes very fine strands. The granules and strands are apparently lipoid in nature. Weissenberg first thought that these granules represented the virus of the disease but later withdrew this opinion. Occasionally fat droplets are found. The network in the large lymphocystic cells surrounds the nucleus and in some places there are clublike thickenings sprouting out. As the cell grows the network extends like latticework. As the network extends, its strands become thickened, increasing in thickness from 3 to 12 microns. In flounders the strands of the network may thicken to from 15 to 25 microns. The network takes the nuclear stain. In places its strands are vacuolated, the vacuoles being acidophilic. In older cells the vacuoles stain more intensively, and according to Weissenberg this may be considered the stage at which degeneration begins. Joseph(10) has shown that later the surrounding epithelial tissue grows into the degenerated lymphocystic cell and entirely fills the space within the membrane. The membrane, however, persists.

The virus of lymphocystic disease and its transmission.—Lymphocystic disease of fishes was mentioned in the literature in 1874 by Lowe(11) and in 1884 by McIntosh.(12) In 1892 Sandemann(13) published a more-detailed description of the disease and advanced the theory that the large peculiar cells were eggs of parasites. This view was later held by Zschiesche(14) in 1910. In 1904 Woodcock advanced the theory that the lymphocystic cells are parasitic protozoans. He designated them *Lymphocystis johnstonei* Woodcock. A few years later Awerinzew, in 1907, confirmed these observations. This author while studying the metamorphosis of the cells during growth noted a peculiar cell inclusion which consisted of a network that stained like chromatin. Awerinzew believed that during the stages of development, dustlike particles of chromatin detached themselves from the nucleus and later arranged themselves into a network. He also thought that the giant cells divided into further secondary amœboid bodies from which he thought spores were produced. In 1914 Weissenberg published a report on lymphocystic disease in which he stated that the disease is due to the intracellular location of a virus that could not be microscopically demonstrated. In 1921 this author published an extensive treatise on the entire subject and confirmed his previous conclusions in regard to its etiology. He states that when healthy and infected bass are placed in an aquarium, the healthy bass become infected and the previously infected fish show new formations of nodules. Furthermore, if the lymphocystic tumors were scraped off, emulsified, and placed in an aquarium containing healthy bass, the disease developed in about two weeks. A possible criticism of such an experiment is, of course, that the fish might have been infected before being placed in the aquarium.

In one experiment Weissenberg transmitted the disease to a bass coming from fresh water. This was accomplished by contaminating the aquarium with an emulsion of the lymphocystic tissue. In another experiment four of six infected fish placed in water to which an emulsion of lymphocystic tissue had been added, developed new nodules. Of five control fish from the same catch placed in fresh uncontaminated water only one developed the disease. The author believes that the virus responsible for the disease is contained in the lymph tumor cells. The disease has appeared as early as nine days under experimental conditions. Weissenberg failed to induce the disease by direct inoculation of lymphocystic material into the skin of fishes. He states that nine days after infection the typical network is not to be found, although the cells are already surrounded by a membrane. Toward the end of the second week of infection a small spherical body differentiates itself within the cell without any relation to the nucleus. This body takes the nuclear stain. It changes into a disklike body and then passes through stages in which it markedly resembles the Guarnieri bodies of the corneal cells of the rabbit inoculated with vaccine virus. This disk later develops into the network body. Weissenberg came to the conclusion that the network body is a specific reaction product comparable to the cellular inclusions of the Chlamydozoa diseases. In 1917 Joseph came to the same conclusion independently while working with lymphocystic disease in a different species, *Sargus annularis*. Joseph also believed that the lymphocystic cells were not foreign but that they are the animal's own elements which hypertrophy as a consequence of an intracellular virus. Joseph, contrary to the opinion of Weissenberg, believes that it is not always the connective-tissue cell that undergoes lymphocystic metamorphosis but that it is the normal organ of the cell, so called "centrophorium," that hypertrophies and becomes more visible. The term corresponds to our present concept of the Golgi apparatus.

Weissenberg, however, has demonstrated quite clearly that the lymphocystic cells originate from the connective tissue. He states that the secondary amœboid bodies described by Awerinzew were most likely degenerated cells invaded by leucocytes.

This author calls attention to the observation by Calmette and Guerin that when vaccine virus is injected into the blood stream of rabbits and the skin is subsequently traumatized, the virus is localized in the skin at the site of the trauma. Gins, it will be recalled, was able to demonstrate Guarnieri bodies within three to seven days following intravenous inoculation of vaccine virus when the cornea was scarified. Weissenberg states that the lymphocystic disease develops earliest and most often on the fins where injuries are most likely to occur. The fact that susceptible fishes may be reinfected indicates that little immunity is produced, and, if any is produced, it is of very short duration.

Microscopically the virus has not been identified. It is without doubt present in the lymphocystic tumors. Weissenberg suggests that the virus rests within the lymphocystic cell and causes its hypertrophy. He states that the tinctorial character of the network is different from that of the Golgi apparatus and that the multiplicity of the original spherical bodies is against the concept that it is a normal development of the cell. This author stands firm in his belief that the network represents a cellular inclusion in that it is similar in many of its stages to Guarnieri bodies. (A network has also been described in the development of the Guarnieri bodies.) Furthermore, in both the lymphocystic network and in Guarnieri bodies there are various sizes of bodies indicating different stages in development.

All attempts to cultivate organisms from the lymphocystic cells have been negative. It may be concluded from the available information that the evidence supports the view that lymphocystic disease is similar in many respects to other filterable virus diseases associated with inclusion bodies. No filtration experiments have as yet been made with the view of experimentally infecting susceptible fishes with this virus. It is to be hoped that such work will be done in the future in view of establishing the true nature of the etiology of this disease.

A lymphocystic disease has been described by Gilruth and Bull, in an Australian kangaroo, *Macropus* species, which is thought to be caused by Sarcosporidia.

BIBLIOGRAPHY

1. KEYSSELITZ, Arch. Protistenkunde 11 (1908) 326.
2. GESSNER (1563). Quoted from Hofer.
3. HOFER, Handbuch der Fisch Krankheiten. B. Heller, München (1904).
4. WIERZEJSKI (1887). Quoted by Hofer.
5. LOWENTHAL, Zeit. Krebsforch. 5 (1907) 197.
6. WEISSENBERG, Handbuch der Pathogenen Protozoen, No. 9 (1921) 1344 (lit.).
7. JOHNSTONE, Rep. Lancash. Sea-fisher. Laborat. for 1904 (1905).
8. WOODCOCK, Rep. Lancash. Sea-Fisher. Laborat. for 1903 (1904).
9. AWERINZEW (1907). Quoted from Weissenberg.
10. JOSEPH (1917). Quoted from Weissenberg.
11. LOWE (1874). Quoted from Weissenberg.
12. MCINTOSH (1884). Quoted from Weissenberg.
13. SANDEMANN (1892). Quoted from Weissenberg.
14. ZSCHIESCHE (1910). Quoted from Weissenberg.

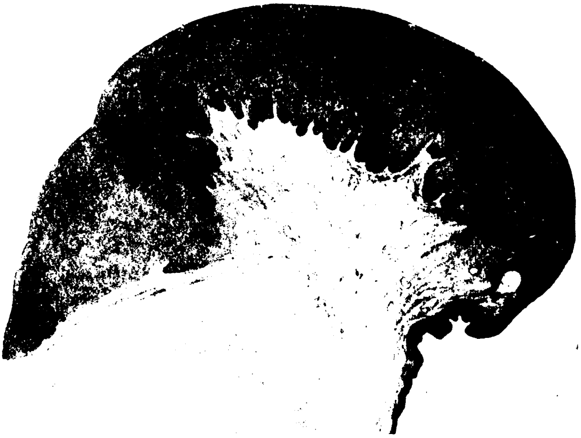
ILLUSTRATIONS

PLATE 40

FIG. 1. Epithelioma of a fish (*Barbus*). (After Keysselitz.)
2. Changes in the nucleus of the epithelial cells in epithelioma; 1 to 9, serial stages; 10, composite picture of a group of stages. (After Keysselitz.)

PLATE 41

FIG. 1. Carp pox. (After Hofer.)
2. Cross section through lesion of carp pox, showing increase in the epithelium. (After Hofer.)
3. Carp pox. *Myxobolus cyprini* with a spore, *sp*, and yellow body, *gk*. (After Hofer.)
4. Section through kidney of carp affected with carp pox; *ep*, epithelial cells of tubules; *par*, parenchyma cells of kidney; *myx*, *Myxobolus cyprini*. (After Doflein; from Hofer.)



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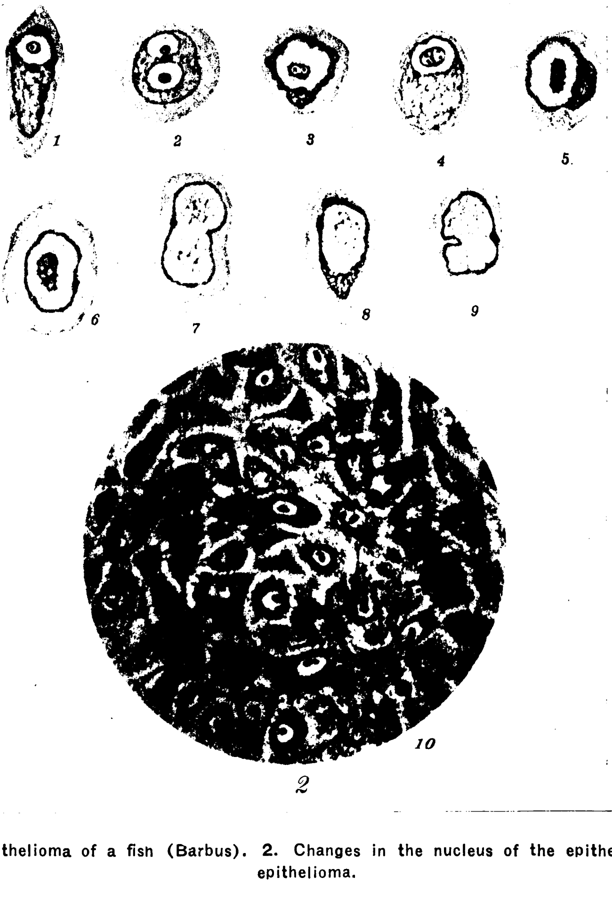

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Fig. 1. Epithelioma of a fish (Barbus). 2. Changes in the nucleus of the epithelial cells in epithelioma.

PLATE 40.

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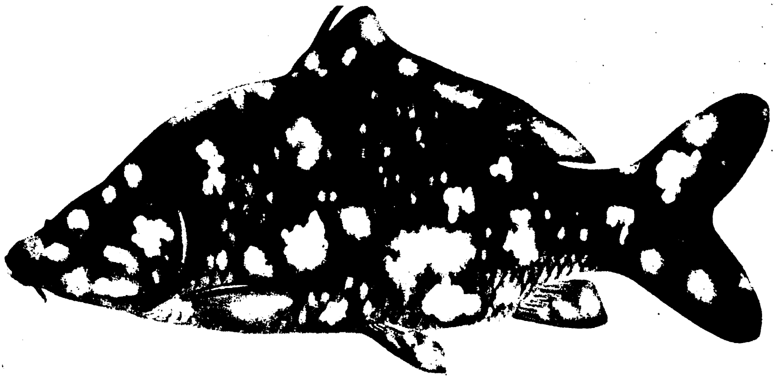

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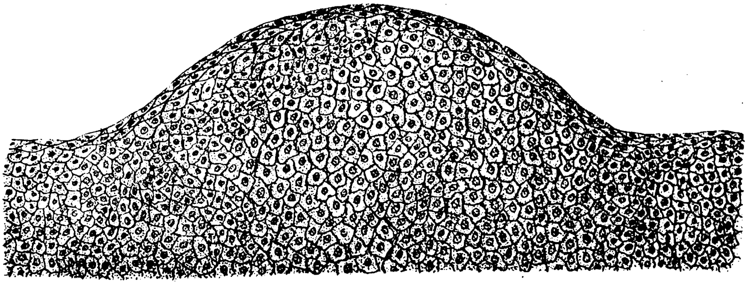

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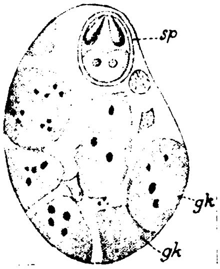

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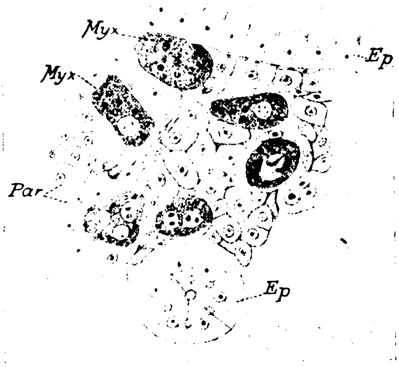

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PLATE 41. CARP POX.

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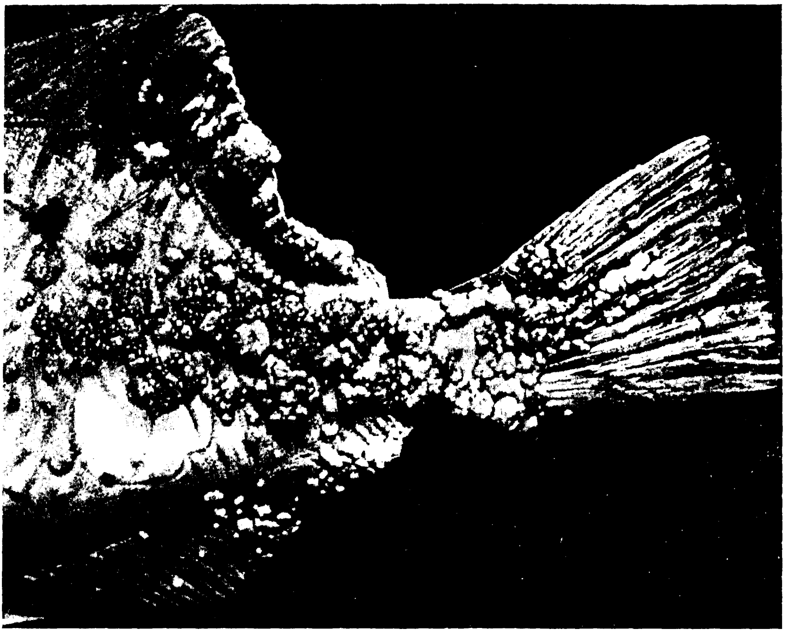

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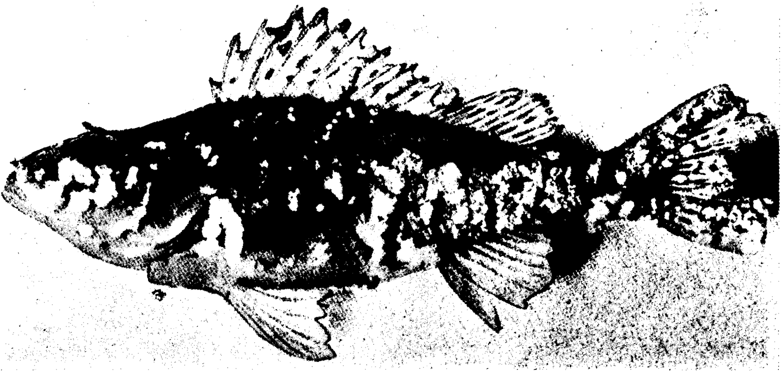

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Fig. 1. Lymphocystic disease of a fish. (After Weissenberg.)
Fig. 2. Bass covered with lymphocystic nodules. (After Weissenberg.)

PLATE 42.

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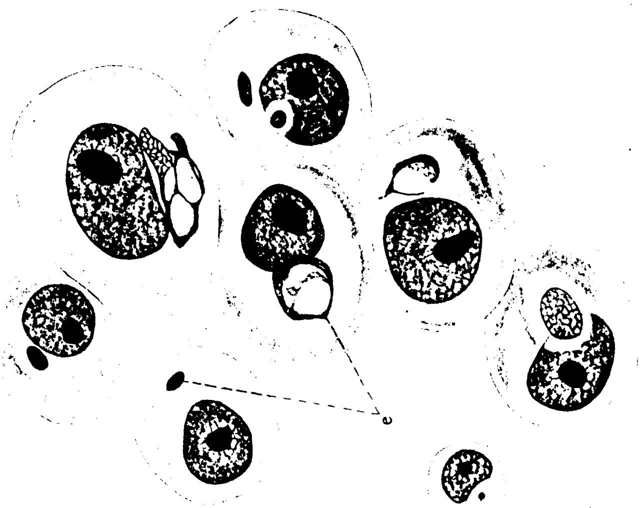

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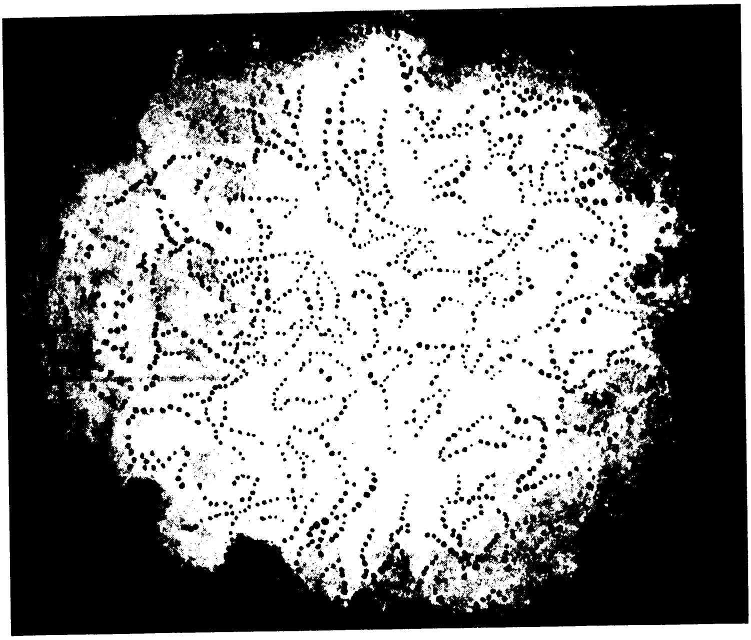

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Fig. 1. Intracellular inclusion bodies within young lymphocystic cells. 2. Cross section of lymphocystic cell of the bass.

PLATE 43.

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PLATE 42

FIG. 1. Lymphocystic disease of a fish. (After Weissenberg.)
2. Bass covered with lymphocystic nodules. (After Weissenberg.)

PLATE 43

FIG. 1. Various stages of develoment of intracellular inclusion bodies within young lymphocystic cells. (After Weissenberg.)
2. Cross section of lymphocystic cell of the bass showing chains of mitochondria in the center. (After Weissenberg.)

CHAPTER XIV

FILTERABLE VIRUS DISEASES OF PLANTS

INFECTIOUS CHLOROSIS

That plants are subject to definite diseases has been recognized from the earliest times. Early writers attempted to explain the various diseases of plants upon the basis of superstition just as the diseases of man and animals were interpreted in this way. The history of the diseases of all forms of life is the history of human fallibility and error. Through the ages there have been magic, superstition, cults, and primitive modes of religious belief. Prior to the time of Plenciz (1762) it was a common belief that disease was transmitted through the air. Swampy land was of particular danger. This was known as the miasmatic theory of the origin of disease. With the diseases of plants as with the diseases of other forms of life we find records attributing these diseases to various superstitions, definite environmental factors, as drying winds, unfavorable soil, and so on. Later when fungi were constantly found to be associated with certain diseases of plants, their presence was regarded only as a special manifestation of the diseased condition such as products of the changed sap of the plant.

Phytopathology as a special branch of study is comparatively recent though its history begins with the civilization of man. An interesting account of the history of plant pathology is contained in a paper by Bailey.(1) Plant diseases such as blightings, rusts, mildews, and blastings are mentioned in biblical literature. These diseases were thought to be an expression of the wrath or disfavor of the Deity. Bailey states:

> The true etiology of disease in plants in these ancient days was for the most part buried deep in the mystery and superstition from which it was a long time in being extricated. Legends were evolved to explain the maladies and diseases were dedicated to special gods, such as the Roman rust gods, Rubigus and Rubigo, in whose honor annual festivals of propitiation were held. From 476 A. D. to the beginning of the seventeenth century, which includes the "Dark Ages," was a dark age for Phytopathology, too, but interest was revived in the seventeenth century, this time among the farmers and agriculturists, whereas previously it was the philosopher who speculated on the cause of disease while the superstitious farmer offered up libations to the gods.

There are still farmers who plant certain crops by the dark of the moon and in accordance with various local superstitions.

In 1660, in Rouen, France, the first law was enacted for the purpose of controlling a plant disease. This law provided for the destruction of the barberry, which was thought to bear some relation to wheat-rust. During the eighteenth century there was an attempt to classify and name the plant diseases. Many of the names adopted were similar to names of human diseases, such as plant cancer, fevers, tumors, etc. Bailey states:

> It must be remembered that all during this time any fungi found associated with disease lesions were considered not as causes of the disease, but as abnormal structures resulting from the disease—that is, morbid plant tissues. Although more emphasis was now laid on the causal nature of such enviromental factors as droughts and freezing, the etiology of plant diseases was still largely assigned to super-natural forces. There was an occasional leaning toward the autogenetic theory of disease, the plant being considered to have within it a disposition to disease. Unger (1883), a strong advocate of the autogenetic theory, believed that fungi originated from the diseased host tissues, but still he recognized them as distinct organisms worthy of names and classification. He believed diseases were brought about through internal disorganization of the nutritional processes, having their origin in a lack of certain chemical constituents of the sap. The fungi, or entophytes as he called them, were the transformed sap of these diseased tissues, the morbid sap being exuded into the intercellular spaces and there converted under the influence of the still living cells of the host into fungus structures.

Later, during the early part of the nineteenth century, mycologists discovered that the fungi were independent structures capable of reproducing their kind by means of spores. It was not until then, however, that the causative relationship of these fungi to disease processes was recognized. In 1853 De Bary demonstrated that certain rust and smut diseases are actually caused by fungi. Bailey writes:

> Perhaps the last great stage in the development of phytopathology as an independent field of work was ushered in by the discovery of Bordeaux mixture in 1883 and the subsequent emphasis placed upon the economic features of plant pathology. . . . the fungicidal properties of Bordeaux mixture were learned quite by accident. Millardet, a young Frenchman, was a physician who abandoned medicine for the pursuit of botany. While attempting to devise control measures with which to combat the devastating spread among the wine grapes of an introduced American fungus-causing mildew, he accidentally observed the prophylactic effects of a mixture of copper sulphate and lime which had been sprinkled on grapevines near the road to prevent stealing of the fruit.

By pure culture methods and experimental infections many fungi associated with diseases of plants were identified, classified, and their causal relationships to disease established. Likewise many diseases were found to be caused by bacterial forms similar to those affecting man and animals. The latter half of the nineteenth century was an important period in medicine. Following the discoveries of Pasteur and Koch new fields were opened for investigation and the foundation of modern medicine and surgery was laid. Hand in hand with developments in medical science were the discoveries and developments in plant pathology. There were, however, a number of diseases affecting plants, as well as man and animals, for which no etiologic agents could be found. These were known as "physiological diseases." These were thought to be caused by some functional or metabolic disturbance. As Smith(2) has stated, they are a heterogenous collection of phenomena, agreeing in nothing except that their cause is not known.

Within the last forty years a voluminous literature has accumulated on the so-called mosaic diseases, a group of diseases which have certain features in common. This group of diseases presents evidence that they are caused by parasites, and there is now general agreement that these parasites fall in the category of the ultramicroscopic viruses, since no demonstrable organisms have as yet been found which cause them. Furthermore, the mosaic diseases of plants have many features in common with the filterable virus diseases found in other forms of life—mammals, insects, birds, and fishes. They are readily communicable, and the sap from the diseased plant is capable of inducing the infection in healthy plants after it has been passed through a bacteria-proof filter. As in several human and animal diseases, the mosaic diseases may be transmitted to healthy plants through the agency of insects.

One of the best-known and most thoroughly studied mosaic disease is the mosaic disease of tobacco. The disease of tobacco manifests itself in various ways. The plant may be entirely dwarfed or may show complete or only partial chlorosis. The leaves may show curling, dwarfing, mottling, blistering, and distortion. In *Nicotiana tabacum* the pink flower may become bleached or blotched with white. The virus of mosaic disease of tobacco has never been seen. It is present in the sap of the diseased plant and may be transmitted to healthy tobacco plants by rubbing the sap upon its leaves. The infectious agent passes through ordinary bacteria-proof filters, but in the case of tobacco-mosaic virus Allard(3) has succeeded in removing the infectious principle by means of the Livingstone atmometer porous cup. The filtrate gives an intense peroxidase reaction, which indicates that the infectious principle is not an enzyme in nature.

Mosaic disease affects a variety of plants. Furthermore, there is thought to be a specificity of the infectious principles found in mosaic-diseased plants of different families, indicating that there are several mosaic viruses. Among the plants that are affected by mosaic are tobacco, tomato, potato, cucumber, lettuce, spinach, sugar cane, turnip, mustard, cabbage, banana, sugar beet, clover, sweet pea, bean, maize, and raspberry. Frequently other plants affected by mosaic disease have been reported.

In this section it is our purpose to describe the mosaic disease of some of the most important of these plants and present some of the fundamental facts that have been learned concerning them. As we have previously pointed out it is important that investigators in the filterable virus field inform themselves of the fundamentals of these diseases as they occur in all forms of life. While this review has emphasized principally the human diseases of filterable virus origin, we have also been interested in similar diseases as they affect animals, insects, birds, and fishes. It is also important that we examine the filterable virus diseases of plants in order that we may determine the common points of convergence in the filterable viruses as they exist throughout nature.

MOSAIC DISEASE OF TOBACCO

We are indebted to Allard(3) for an extensive study of the mosaic disease of tobacco. The work of this investigator has resulted in far-reaching conclusions in regard to the etiology of this disease. In one of his publications Allard states: "Mosaic is one of the most serious and widespread diseases known to affect the tobacco plant." This disease is known locally as "calico," "gray-top," "mottled top," "mottling," and "foxy." In certain localities the terms "walloon," "chlorosis," "brindle," and "mongrel" are also used.

The mosaic disease of tobacco is of particular interest because the first work on this disease, by Iwanowski,(4) is usually considered the corner stone of the study of all the filterable viruses. In 1892 Iwanowski discovered that filtrates of tobacco mosaic remained active for several months at ordinary temperatures. These early observations of Iwanowski were later independently confirmed by Beijerinck,(5) who advanced the theory of the possible existence of a "contagium vivum fluidum." In 1900

Heintzel(6) and in 1902 Woods(7) independently concluded that mosaic disease of tobacco is caused by oxidizing enzymes. Hunger(8) in 1905 published a treatise on the disease in which he refuted the theory of Heintzel and Woods that mosaic of tobacco is due to enzymes, and suggested that the disease is brought about by certain unfavorable conditions of growth whereby toxins that lead to the appearance of the disease are produced within the plant. In 1914 Allard reported convincing evidence that mosaic disease is not of physiological origin but that it is caused by a specific infection. Allard states:

> Both in Europe and America the mosaic disease of tobacco has been the subject of wide inquiry. Hitherto no investigator has been able to offer conclusive evidence which would consistently explain the baffling nature and mysterious origin of the disease. Each in his own way, however, has emphasized a favorite opinion, so that the literature of the disease is especially conspicuous for its widely contrasted theories. The view seems to be generally accepted that the disease is a physiological or functional disorder, although it has long been known that it is more or less infectious. For this reason the writer's experiments were at first planned along physiological lines. Facts soon came to light, however, which led to the conclusion that the disease must be parasitic in its origin rather than physiological or functional.

In regard to the symptoms of mosaic in tobacco Allard says that the following symptoms are more or less characteristic of different phases of the disease at one time or another: Partial or complete chlorosis; curling of the leaves; dwarfing and distortion of the leaves; blistering or "savoyed" appearance of the leaves; mottling of the leaves with different shades of green; dwarfing of the entire plant; dwarfing and distortion of the blossoms; blotched or bleached corollas (in *Nicotiana tabacum* only); mosaic sucker growths; and death of tissues (sometimes well marked in *Nicotiana rustica*). The character of the symptoms depends largely upon the age and vigor of the plants when they are infected.

In young plants affected with mosaic downward curling of the leaves is one of the first symptoms noted. Later, depending upon the conditions under which the plants are raised, there develop blisters or the so-called "savoyed" appearance and mottling. As the plant approaches maturity the symptoms known as "mottled top" or "gray-top" develop. As the disease develops, a great variety of changes are produced in the leaves of the plant. The blotched or mottled appearance of the leaves is characteristic of the disease. Distortions and irregularities of growth are not unusual. Plants vary in their response to the virus of mosaic. Some are apparently more resistant than others. Allard believes that the examination of the blossoms affords one of the surest indications of the presence of the disease in a tobacco plant. He says:

> As a rule, in blossoms of mosaic plants the normal pink coloration is present only in lines, specks, or conspicuous blotches. In most instances these markings are very irregular in distribution, sometimes involving a portion of all of the lobes of the corolla. A rather striking and symmetrical color pattern is sometimes afforded by a blossom in which the normal pink coloration occurs only as a fine line in the sinus of each corolla lobe. Other affected plants produce blossoms all of which are devoid of color, so that they appear quite white or very pale.

In malignant forms of the disease the plants often produce depauperate and misshapen blossoms. Apparently these observations hold only for the pink-flowered varieties of *Nicotiana tabacum.* Allard has not observed these symptoms in the green, purplish yellow, red, or white-flowered species of *Nicotiana.*

The symptoms of mosaic do not appear in all parts of the plant at the same time. The symptoms may be localized or general. In the former case the only lesions may be a few scattered blisters upon an otherwise apparently healthy leaf. As the disease progresses it invariably becomes general in that it appears in other immature growing parts of the affected plants. Local lesions may be produced in a number of ways, but the symptoms of the disease depend on many other factors, such as the resistance of the plant, its vigor, and environmental conditions. Allard was able to transmit the mosaic disease of tobacco to a great variety of solanaceous plants, but his efforts to communicate the disease to plants of other families always gave negative results. From his transmission experiments he concludes that several species appear to be immune to the disease. Furthermore, in the susceptible group some species show a greater resistance to the infection than others. Allard inoculated the following plants with tobacco mosaic, and symptoms of the disease developed:

Nicotiana (all varieties of *N. tabacum* tested and many distinct species of *Nicotiana*).
Lycopersicon (several of the more distinct varieties of tomato).
Petunia violacea.
Physalis (two distinct garden species).
Datura stramonium and *D. tatula.*
Hyoscyamus niger.
Solanum nigrum and *S. carolinense.*
Capsicum (several of the more distinct varieties).

Of the species of *Nicotiana* inoculated with tobacco-mosaic virus Allard was able to produce symptoms in *Nicotiana silvestris, N. rustica, N. longiflora, N. alata, N. plumbaginifolia, N. forgetiana, N. paniculata,* and *N. langsdorffii.* He was unable to infect *N. glauca* and *N. viscosum.*

The incubation period in mosaic disease of tobacco is variable. It depends upon several factors, among which are the age of the plant, the kind of plant, and various external factors that may retard or accelerate growth. Woods reported eight days between the time of infection and the appearance of the first symptoms of the disease. This is one of the earliest periods yet reported. In Allard's experiments the shortest period between inoculation and the appearance of the first symptom of the disease was six to seven days. Mayer(9) has reported an incubation period of ten to twelve days for the disease. On the average of many hundreds of experiments Allard believes that twelve to fifteen days is the usual period of incubation.

According to some authors mosaic disease of tobacco sometimes disappears either spontaneously or under treatment. Both Beijerinck and Woods stated that under certain conditions plants sometimes appeared to recover from the infection. Lodewijks(10) reports that he has been able to effect a cure of the diseased plants by treatment with blue light. By exposing a healthy part of the plant this author claims that an "antivirus" is produced that destroys the virus of the disease. Allard states that covering the affected leaves of the plant would reduce the color contrast of the mottled areas and that Lodewijks may have mistaken this for cure. In thousands of plants experimentally infected by Allard there has not been a single recovery. Gile(11) reported the use of solutions of iron salts which he applied to the leaves of infected plants. He states that such salts and crystals of ferrous sulphate were effective in overcoming pineapple chlorosis in Porto Rico. In Allard's experience this method of treating mosaic disease of tobacco has been without beneficial effect. In general a rapid and extensive development of symptoms is coincident with rapid growth according to Allard. Any conditions that retard growth will, it follows, prevent the further appearance of mosaic leaves and branches.

Allard has shown that the excision of all portions of the plant showing mosaic symptoms does not rid the plant of the disease. Symptoms appear later in the new growing portions of the plants. In some instances the excision of infected parts appears to hasten the development of the disease in other parts. He states: "Plants showing mosaic symptoms only in the topmost leaves or even in the blossoms alone may be cut completely to the ground, yet mosaic symptoms, often of the most malignant character, again appear in the shoots which arise from these stubs."

The virus of mosaic disease of tobacco.—The sap from mosaic-infected tobacco plants remains infective after passing through a bacteria-proof filter. This has been demonstrated by Iwanowski, Beijerinck, and Allard and repeatedly confirmed by other investigators. Ground and dried mosaic material remains active for over a year and a half. The virus was found by Allard to be preserved for four months in ether, toluene, and glycerin. Filtration of the mosaic-infected juice through Berkefeld and Chamberland filters is followed by some loss in infectivity. Its infectivity is entirely lost when filtered through the Livingstone atmometer porous cup. In the latter case, however, the filtrate gives an intense peroxidase reaction with guaiac and hydrogen peroxide. When filtered through powdered talc under three inches of mercury Allard found that the talc, by absorption, removed all of the peroxidase from the pure virus. By reducing the amount of talc the peroxidase content may be increased until limits are reached beyond which the infective principle also passes into the filtrate. Some authors have found that the first portions filtered give an intense peroxidase reaction but are not infectious, while later portions contain the infectious material. Using the Hirsh porcelain funnel with perforated disks covered with filter paper to retain the talc, and filtering under reduced pressure of three inches of mercury, Allard found that the virus of mosaic is held back while the filtrate gives a strong peroxidase reaction.

Eighty per cent alcohol destroys the virus in thirty minutes, but the material still gives a strong peroxidase reaction; 45 to 50 per cent alcohol does not destroy the virus for several days. However, the infectious agent is carried down in the precipitate with weak concentrations of alcohol leaving the supernatant non-infectious. The supernatant still gives a strong peroxidase reaction. Chodat and Bach(12) have shown that the oxygenase in the sap of a species of *Lactarium* is largely precipitated by 40 per cent alcohol while the peroxidase remains in solution.

The peroxidase precipitated by strong alcohol from the sap of mosaic plants is not capable of producing the infection in healthy plants.

Schönbein(13) has shown that while peroxidase activates small amounts of hydrogen peroxide, large amounts of hydrogen peroxide will destroy the peroxidases. Hydrogen peroxide will then destroy the peroxidase without destroying the infectious properties of the sap. Chodat has demonstrated that for constant quantities of peroxidase, the oxidation products increase directly with the amount of hydrogen peroxide present, within limits, until all the peroxidase is combined or used up. If the virus evolves little or no oxygen upon addition of hydrogen peroxide, a very small quantity of this reagent destroys the peroxidase.

According to Loew(14) the peroxidase of tobacco is unaltered in a 5 per cent solution of formaldehyde after forty-eight hours. Kastle(15) has found that oxidase of the mushroom, *Lepiota americana,* is not destroyed by a 40 per cent formic-aldehyde solution after several days. In Allard's experiments the virus of mosaic disease of tobacco retains its infective properties in a solution which contained one part of formaldehyde in one thousand parts of virus after thirty-two days. The mosaic virus is destroyed by ethyl and methyl alcohol, by aluminum hydroxide, by temperatures near the boiling point of water, but not at 80° C. when kept at this temperature for a few minutes. In some instances five-minute exposures at this temperature are sufficient to destroy the virus.

Allard says:

> The writer's experiments show that peroxidase or catalase in the sap of mosaic plants cannot be responsible for the mosaic disease. The same enzymes are normally present in healthy plants, but the sap of such plants is without infectious properties. By evaporation the enzymes present in healthy sap may be brought to a high concentration, and such solutions never acquire infectious properties. By dilution, on the other hand, the peroxidase content of mosaic sap may be diminished to such an extent that peroxidase reactions are no longer discernible; yet such solutions may remain highly infectious. He further concludes that since it has been shown that the mosaic disease of tobacco does not occur in the absence of infection, neither enzymes nor other normal constituents in the sap of healthy plants can be considered responsible for the disease. A specific, particulate substance not a normal constituent of healthy plants is the cause of the disease. Since this pathogenic agent is highly infectious and is capable of increasing indefinitely within susceptible plants, there is every reason to believe that it is an ultramicroscopic parasite of some kind.

According to Allard, embryonic transmission of mosaic disease has not been observed in tobacco plants. There is evidently some barrier that guards against embryonic infection.

Early in the course of his work on mosaic disease of tobacco Allard discovered that the disease may be transmitted from infected plants to healthy plants through the agency of aphids. He believes that aphids may sometimes be responsible for the occurrence of the disease in the seed bed and its subsequent spread in the field.

The virus of mosaic is active in very high dilutions. In one experiment Allard inoculated a group of young plants in three-inch pots in the greenhouse by placing a drop of diluted sap, 1 : 10,000, with the point of a needle upon each leaf. The disease was readily produced, the incubation period ranging from seventeen to twenty-five days in this particular experiment. Infection may be produced with higher dilutions than this, but even this dilution is strong evidence against the theory that the disease is produced by enzymes since the actual amount of virus in one drop of such a dilution must be exceedingly small.

It is evident from the work of Allard that the infectious principle is not an oxidase since the infectious principle and the oxidizing enzymes react independently. Freiberg(16) has confirmed this work but believes that the incitant is an aldehydase. This author found that the carbohydrate content of the dark-green is higher than of the light-green areas of the leaf, and believes that formaldehyde is one of the first products of photosynthesis, and that the infective principle is destroyed by a specific chemical reaction with formaldehyde and not by its antiseptic properties.

It has been pointed out by Wakefield(17) that the behavior of the virus of mosaic disease of tobacco with respect to high and low temperatures and various antiseptic solutions is more in accord with that of an enzyme than with that of any organism at present known. However, this author further states:

> On the other hand, the great objection to the enzyme theory appears to be the difficulty of explaining how such a catalyst would originate. If one adopts this view, it is necessary to assume that at some period an enzyme, or a substance capable of activating enzymes, has arisen in the plant *de novo*—possibly, as Freiberg suggests, as a consequence of some disturbance in metabolism. Were this the case, it should be possible to induce the disease at any time, by engendering such a disturbance in the activities of the plant cell.

The fact that the virus is active in very high dilutions and increases within the tissues of the plant points to a living organism rather than an enzyme.

That the virus of mosaic disease of tobacco is exceedingly small is indicated by filtration experiments. Duggar and Karrer(18) state that the virus of tobacco mosaic is approximately the size of colloidal particles of hæmoglobin, which has been determined to be about 30 millimicrons. This is also about the estimated size of bacteriophage particles. (D'Herelle.)

The virus of tobacco mosaic has within recent years been found to be pathogenic for both tomato and potato plants.

MOSAIC DISEASE OF TOMATO AND POTATO PLANTS

The tomato plant is susceptible to mosaic virus as are the fruits of the pepper, cucumber, and bean. Mottling is one of the most important affections of the tomato. These fruits are also affected by russeting or spotting, dwarfing, and distortion.

Tomato mosaic occurs very commonly in hothouse tomato crops according to Gardner and Kendrick,(19) and these authors believe that the disease may be carried to the field crops from the hothouse. They state, however, that this does not account for the great bulk of mosaic infection in the canning crop. They were unable to find any evidence of seed transmission. In Indiana these authors have found mosaic occurring on several perennial weeds such as *Physalis subglabrata, P. virginiana, P. heterophylla,* and *Solanum carolinense,* and the disease has been transmitted to tomatoes from each of these species. Aphids and flea beetles may also transmit the disease from *Physalis* to tomatoes.

Olitsky and Northrop(20) have been able to transmit mosaic from mosaic potato plants to tomatoes and to tobacco. The signs of the disease in tomatoes and tobacco are identical. These authors also found that centrifugalization of the virus derived from tobacco, potato, or the tomato at 3,200 revolutions per minute for two hours did not throw down the virus and the supernatant fluid induced the infection as quickly, actively, and constantly as the sediment.

Dickson(21) also found that healthy tomato plants inoculated with a mixture of viruses from mosaic-diseased tomato and potato, or tobacco and potato, developed streaks in about fourteen days. This author, however, found that bean-mosaic and raspberry-mosaic viruses with tomato-mosaic virus gave negative results. He believes that the so-called Quebec streak or stripe of tomato is caused by a double infection with the viruses of potato and tomato mosaic, tobacco mosaic in this case being considered the same as tomato mosaic.

Mosaic disease of potatoes has been thought by some investigators to be caused by the same virus that affects tobacco. Johnson(22) states that tobacco mosaic produces brown or black necrotic lesions on the stems and petioles of potatoes at the point of inoculation but that tobacco-mosaic infection is not found to be systemic in the potato and that the potato cannot be said to be a typical host of tobacco mosaic. Schultz(23) has described in detail the mosaic disease of the potato. Previous descriptions were also published by Melchers,(24) Melhus,(25) Murphy,(26) and Stewart.(27) Schultz states:

> On Green Mountain or Bliss Triumph potatoes, the leaves of affected plants are characterized by mottling which is produced by the presence of light green areas on the foliage. These areas may occur on any part of the leaf; they may include or adjoin sections of the larger veins or not come in contact with them. The light green patches vary greatly in shape, being punctate, elongate, circular, angular, and irregular. . . . Their dimensions seldom exceed a few millimeters. . . . Furthermore, in the more advanced stages the foliage presents a characteristic crinkled or corrugated appearance. In these stages the diseased plants are frequently dwarfed because the stems, the leaf petioles, and leaf blades are considerably shortened or reduced in size. The symptoms as described above are not so marked in certain other varieties—for example, in Blue Victor, Early Rose, Irish Cobbler, Pearl, White Bliss, Carmen, Early Dix, Netted Gem, Peach Blow, Portuguese Purple, and Spaulding Rose. In the first five named, decided rugosity is a characteristic of the disease.

No symptoms have been discovered by which mosaic can be recognized in the dormant tubers. That mosaic plants are produced from infected tubers is now certain. Schultz has shown that potato mosaic may be transmitted by transferring juice from a diseased plant to a healthy plant, by grafting, by at least two species of aphids, and by infected tubers. According to this author the symptoms of potato mosaic are modified by various climatic and enviromental conditions.

With regard to the effect of tobacco mosaic on potatoes there appear to be two different manifestations. The black necrotic lesions of Johnson have already been mentioned. Fernow(28) has reported symptoms similar to the streak of potatoes described by Schultz and Folsom.(29) Blodgett(30) states in this connection:

> The author's results agree with those obtained by Johnson and with those obtained by Fernow. The seeming difference arose from the fact

that Johnson had used Bliss Triumph potatoes for inoculation with tobacco mosaic, while Fernow had used Green Mountains. On Bliss Triumphs, local necrotic lesions were produced at the points of inoculation, with no systemic infection. On Green Mountains the symptoms were much like those described for streak, consisting of streaks on the stems and veins and necrotic spotting on the leaves.

It is apparent from the above discussion that the mosaic virus of tobacco is pathogenic for both potatoes and tomatoes and vice versa. There is apparently a close relationship between these viruses if, indeed, they are not identical.

MOSAIC DISEASE OF CUCUMBERS

In 1902 Selby(31) in Ohio and in 1910 Stone(32) in Massachusetts reported a mosaic disease on the leaves of cucumbers grown in the greenhouse. Clinton(32) in 1908 noted chlorosis of muskmelon leaves in Connecticut. In 1916 Gilbert,(34) Jagger,(35) and Doolittle(36) demonstrated the infectious nature of this disease for cucumbers, although Selby had stated in 1910 that cucumber mosaic was transmitted like that on tobacco. The cucumber mosaic is widespread throughout the United States.

Doolittle in 1920 reported his extensive work on this mosaic disease. This author has demonstrated that the virus is filterable and that the filtered juice from diseased plants is capable of inducing the infection in healthy plants. The disease appears both in the field and in the greenhouse, and nearly all cultivated cucurbits are susceptible. The diseased plants show a yellow mottling on the leaves and also a wrinkled or savoyed appearance. The older leaves turn yellow and die. The stems terminate in clusters of dwarfed leaves. Mosaic fruits of the cucumber are mottled with green and yellow and frequently develop wartlike growths. Doolittle says:

> Nearly all species and varieties of the genera Cucumis, Cucurbita, Lagenaria, Luffa, Momordica, Trichosanthes, Ecballium, Benincasa, Micrampelis, and Sicyos are susceptible to the disease, but the Citrullus species seem to be partially resistant.

No visible causal organism has been associated with cucurbit mosaic. The mosaic virus is destroyed when heated above 70° C. It is also destroyed by formaldehyde, phenol, and copper sulphate in 0.5 per cent solution and by mercuric chloride in a 1: 2,000 dilution. Ten per cent chloroform also destroys the virus, but 5 per cent is apparently harmless. The virus is contained in the juice of the diseased plant and remains infective even when diluted to 1 : 10,000. The infectious agent passes through the pores of a Berkefeld filter but is held back by the Chamberland filters. The virus remains infectious only for twenty-four to forty-eight hours in expressed juice and is readily destroyed by desiccation.

The mosaic diseases of tobacco, tomato, bean, potato, and pokeweed do not affect the cucumber. Mosaic of cucumbers is transmitted by insects such as the melon aphis and the cucumber beetle.

Doolittle states that the wild cucumber is affected with a mosaic disease identical with that on the cucumber and suggests the importance of wild hosts as a means of overwintering the mosaic disease. With regard to the incubation period he states that young cucumber plants may show the first visible signs of the disease within four or five days after inoculation and rarely later than eight or nine days. In older plants the disease appears within ten to fourteen days after inoculation.

Elmer(37) states: "It is generally held that mosaic of the Cucurbitaceae, Solanaceae and Leguminoceae are all quite specific within the same family and with few exceptions transmissible only to species within the same family." This author reports that he has been able to produce cross-infections between these families. He states:

> Cross-inoculation experiments by the writer have shown that the mosaic diseases of the Cucurbitaceae, Solanaceae and Leguminoceae are inter-transmissible. Four petunia plants inoculated with mosaic from crookneck squash became infected while an equal number of checks remained healthy. . . . four crookneck squash plants were inoculated with mosaic from tomato, and four with mosaic from tobacco. All of these plants became infected. Similarly a tobacco and two tomato plants were inoculated with juice from mosaic crookneck squash leaves and became infected. All checks remained healthy. . . . At the same time five tobacco plants were similarly inoculated with mosaic cucumber tissue and one of the five became infected. . . . An attempt to inoculate tomatoes with mosaic from catnip, Nepeta cataria, resulted in three of the five plants inoculated becoming infected while an equal number of checks remained healthy.

Elmer believes that infection with mosaic is to a large degree determined by the growth condition of the plant. From his work, if carefully confirmed by others, it must appear that the various mosaic viruses are very closely related. To what degree intertransmission of mosaic virus occurs under field conditions between the different families should of course be determined. Without doubt the virus of mosaic overwinters in some plants in the field, just as it is known to overwinter in the greenhouse. In many instances the infection is present in the seed bed when planting is begun in the spring.

MOSAIC DISEASE OF LETTUCE, CABBAGE, MUSTARD, TURNIP, AND SPINACH

In 1921 Schultz(38) described a transmissible mosaic disease of Chinese cabbage, mustard, and turnip. During the same year Jagger(39) reported a mosaic disease of lettuce.

The mosaic disease of Chinese cabbage, mustard, and turnip is characterized by a distinct mottling of the leaves which is very similar to that of mosaic disease of the Solanaceæ. The mottling is seen as light green and dark green areas on the leaves. Also there is a characteristic ruffling and distorting of the leaf surface. According to Schultz, the dark green patches appear on the raised areas. He states that "The leaf margins frequently are much more irregular than in healthy plants, causing some of the leaves to appear somewhat unsymmetrical. In addition to these common abnormalities on the leaves the entire plant may be dwarfed, and the flower stalk and number of blossoms may be considerably reduced." Schultz was able to transmit this disease to healthy plants when juice from diseased plants was introduced into plants of the same or related species. He was unable to infect these plants with mosaic virus taken from mosaic potato. The incubation period in experimentally inoculated plants was found to range between twenty and thirty days which is very close to the incubation period of mosaic disease in the Irish potato. The disease is also transmitted from diseased plants to healthy plants by aphids as is the case with tobacco mosaic, spinach blight (according to McClintock and Smith(40)), and mosaic disease of potatoes. The mosaic disease of the crucifers, however, was not transmitted to the morning-glory by aphids which transmitted mosaic to healthy crucifers. The plants inoculated by means of aphids developed the mosaic symptoms only on the younger leaves; this was also the case in experiments in which the virus was inoculated by rubbing. Mustard seed from mosaic mustard plants developed healthy seedlings according to Schultz.

Jagger studied mosaic disease in Romaine lettuce and in a variety of head lettuce. In lettuce the symptoms of mosaic begin as a yellowish discoloration along the smaller veins of the younger expanding leaves. Gradually the entire plant becomes yellow. On close examination irregular blotches of a normal green color are found located along the larger leaf veins. The diseased leaves also show wrinkling. The blotching was found by Jagger to be more pronounced on Romaine lettuce than on head lettuce. Generally the diseased plants are stunted. Jagger says:

> In severe cases the plants were decidedly undersized, and occasionally the leaves formed only a rosette, with no indications of a folding together of the tips to form a head. . . . Often plants that showed marked discoloration, mottling, and stunting soon after becoming diseased would later seem to recover in part and to make a more or less normal growth with only slight discoloration and mottling.

Jagger successfully transmitted the disease by aphids from diseased plants to healthy plants, and from the symptoms and general character of the disease he believes that it should undoubtedly be recognized as a true mosaic disease of lettuce.

Mosaic disease of turnips is readily produced experimentally by artificial inoculation. The symptoms of the disease are in general those described for mosaic of other plants. Following artificial inoculation Gardner found that healthy turnip plants developed mosaic within sixteen days. Raddish plants inoculated with the same virus by this author remained negative.

MOSAIC DISEASE OF SUGAR CANE

Mosaic disease of sugar cane was apparently first noted in Java in 1890, where it was known as "yellow stripe," but it was not thought to be infectious by the Dutch. The disease had undoubtedly been present for years in Java but little attention was given it. In 1909 Dutch investigators reported yellow stripe in Egypt on cane imported from Java, and it was noted a year later in the Hawaiian Islands. In 1916 the disease was first seen in Porto Rico. There it has been called matizado, "mottling;" rayas amarillas, "yellow stripe;" morida de perro, "dog bite;" la enfermedad de Arecibo, "disease of Arecibo" (because it originated in Porto Rico in the neighborhood of Arecibo), and by many other names. The disease was first noted in the United States just prior to 1919. It was then discovered in Porto Rico on young cane which had been received from the United States. Since 1919 the disease has been found in many parts of the United States, the Philippines, Cuba, and other parts of the world.

In 1919 Brandes (41) published an extensive paper on mosaic disease of sugar cane. This author describes the symptoms of sugar-cane mosaic as primary and secondary. He states:

> Upon walking between the rows of cane in an affected field, more or less plants will be seen that are conspicuous on account of a general pallor of the leaves. This may be discernible for many rods. Closer examination of such plants reveals that the pallor is due to irregular light-colored streaks or spots on the leaves. The affected leaf areas, in so far as color is concerned, are of two distinct types. The most common type presents merely a "washed-out" appearance. . . . In the second type, the yellow is predominant, and the affected areas have a decided yellowish green appearance. The normal and affected areas are sharply demarked. In other words there is a gradual merging of one color into the other.

In young leaves of ribbon cane the light areas are found as short narrow streaks that may run together at the ends and appear very long. There are many variations. In older leaves the streaks are confluent and the general effect is one of pallidness or yellowness. The disease is never fatal during the first year. The more serious effects are in first ratoons of cane which became infected the previous year or in plants coming from diseased cuttings. In these plants the symptoms consist of white opaque spots and streaks. About 20 to 30 per cent of the entire surface is involved. These areas remain firm and do not rot out. Later another symptom of mosaic appears which consists of stripping or cankering of the stalk. These signs appear as discolored or water-soaked patches or longitudinal streaks on the internodes. In some instances these areas become depressed. Cracks resulting from the drying out of the cane may appear. Brandes says:

> When a large portion of the plants in a field are infested, the aspect in general resembles the effect of a severe drought. The foliage of the entire field is yellowish, and the plants are more or less noticeably stunted. Where a row of some immune variety is planted in or near the badly infested field, the contrast in color is exceedingly conspicuous and the dwarfed habit of infected plants is more noticeable. It is possible to recognize such fields from a distance of half a mile or more on account of their sickly, dry appearance.

There are many degrees of involvement in sugar-cane mosaic. Some species of sugar cane are markedly resistant, while others are exceedingly susceptible to the virus. The writer has seen mosaic on several varieties of cane in the Philippines and in Porto Rico where the disease is generally wide spread. The Japanese canes are as a rule immune to the virus. This canc is also known as the North Indian type. The Kavangire variety is also immune and is found in Argentine, Porto Rico, and other places. This variety has never been found infected and has grown side by side with other canes in Porto Rico which were heavily infected. There are many other hosts for the mosaic virus of sugar cane, such as corn, sorghum, rice, millet, crabgrass, foxtail, and *Panicum.* The virus is the same for all of these plants according to Brandes.

It is now well recognized that cuttings from infected stalks invariably give rise to infected plants. The young shoots are seen to be infected as soon as they appear. These are considered primary infections. As the cane develops secondary infections take place and these are thought to be due to some carrier, perhaps an insect, since insects are known to transmit other mosaic diseases, such as the mosaic of tobacco, tomato, and potato. Brandes states that field disease is accompanied or preceded by severe insect infestation. The cane leafhopper, *Tittigonia* species, has been noted to be particularly prevalent during the spread of the disease. Brandes has established the fact that *Aphis maidis* is able to transmit sugar-cane mosaic. Ledeboer(42) in Java transmitted the disease with *Aphis sacchari* as well as *A. maidis.* This has been further confirmed by Bruner(43) in Cuba and by Kunkel(44) in Hawaii.

Edgerton and Taggart(45) believe that the disease in many instances will tend to disappear if allowed to take its course. They have observed that the susceptible varieties tend to be eliminated while the stronger plants survive and that gradually less and less of the disease is seen under these conditions. They do not advocate elimination of control measures where these are possible but suggest this doctrine which might be termed "the survival of the fittest."

Sugar cane itself is a perennial, and the mosaic virus survives the winter in the stubble. The virus can be recovered from the stubble, and healthy plants can be experimentally infected either by direct inoculation or by insect carriers. Control of this disease by destroying the stubble has been suggested and offers decided assistance, but there are many other hosts for the sugar-cane or grass mosaic and it is difficult to destroy all of these. Brandes and Klaphaak(46) have reported at least thirteen species of grass that are susceptible to the grass mosaic as indicated by experimental inoculation. All species tested for seed transmission by this author gave negative results.

Brandes has been able to transmit the disease to healthy plants with juice expressed from diseased plants. The early Dutch investigators uniformly failed in this. Stevenson(47) reported hundreds of inoculations in several varieties of cane with several methods, but his results were entirely negative. Brandes expressed the infected juice under mineral oil and by careful inoculation produced the disease in susceptible healthy plants. He states:

> That cane mosaic is analogous with other mosaic diseases is brought out by a number of facts, aside from the visible signs of the disease. As in many other mosaics, the infectious material does not seem to be highly specialized, but may attack other plants in the same family. The cell sap of infected plants contains some organism, not visible by ordinary means, which is capable of inducing the infection when injected into healthy plants. Leaves which are mature at the time of inoculation never show any signs of the mosaic. This fact, typical of all mosaics, has been brought out in all inoculation experiments with sugar cane.

While the properties of the virus of sugar-cane mosaic are not as well known as those of tobacco mosaic, it appears to be evident that in sugar-cane mosaic we are dealing with an ultramicroscopic virus which is quite similar to mosaic viruses found in other plants. More information bearing upon the nature of the virus itself is very much needed and should receive attention in future investigations on this disease.

MOSAIC DISEASES OF OTHER PLANTS

Infectious chloroses have been described for a large number of hosts. Among these may be mentioned the banana, sugar beet, bean, maize, raspberry, privet, laburnum, ash, European mountain ash, hop tree, and Japanese burning bush. Mosaic disease is a type of chlorosis. Clinton(48) has classified the various types of chlorosis as follows:

1. Infectious chlorosis.
    - A. Communicable through the juice.
    - B. Communicable through the tissues.
        - *a.* By buds.
        - *b.* By grafts.
2. Noninfectious chlorosis.
    - A. Nonperpetuating.
        - *a.* Affecting plants generally.
        - *b.* Affecting isolated leaves or branches.
    - B. Perpetuating.
        - *a.* Through seeds.
        - *b.* Through cuttings.
        - *c.* Through buds or grafts.

The mosaic diseases are infectious and are communicable through the juice. Because of the mottling on some species the term "infectious mosaic" has been employed. In the majority of horticultural variegated varieties of plants, such as the crotons, oleander, and *Pittosporum,* chlorosis is noninfectious and cannot be communicated either with plant juices or by grafting. The true mosaic diseases are transmitted through the juice, and the infectious principle has been demonstrated in the juice of many plants. In no case of infectious chlorosis is the virus transmitted to the embryo plant in the seed. (See Heald's Manual of Plant Diseases.)

From the original mosaic disease of tobacco numerous mosaic diseases are now known in other species of Solanaceæ, Curcurbitaceæ, Leguminoseæ, and Gramineæ as well as in more than twenty additional families. Rankin(49) and Bennett(50) have recently published extensive reports on mosaic of raspberries. In general these studies indicate that the mosaic disease of raspberries is similar to mosaic in other plants.

Mosaic disease is apparently widespread in nature and affects plants of many families. For the present, we must regard these diseases as being caused by ultramicroscopic viruses.

CAUSAL AGENCY IN MOSAIC DISEASES

Several theories have been advanced to explain the causal agency in mosaic diseases; namely, the bacterial theory, the enzyme theory, the filterable virus theory, and the protozoan theory.

In general it may be stated that the bacterial theory is without foundation. None of the advocates of this theory have presented any experimental evidence that will permit serious consideration of it. The enzyme theory is supported by the fact that the infectious principle is absorbed by talc as is characteristic of colloidal compounds, including enzymes, and by the reaction between the infective principle and formaldehyde and by the resistance of the virus to antiseptics. The infectious substance is also destroyed by concentrations of alcohol that inactivate enzymes and by the same temperatures that are destructive to enzymes. On the other hand most of the investigators who have studied mosaic point out that if the infectious principle is an enzyme it will be necessary to assume that enzymes have the power to initiate the production of the same enzyme when introduced into the normal plant cell, since the infectious substance is propagated indefinitely in susceptible plants. Most investigators are unwilling to admit this. In this respect we have a close analogy in the bacteriophage of d'Herelle.

The filterable virus theory has been fully discussed at the beginning of this section. There seems to be abundant evidence to support this concept and most investigators are inclined to this view.

The protozoan theory has within recent years attracted great attention. A number of early workers described amœbalike bodies in the cells of mosaic tobacco plants. Recent workers have found plasmalike bodies in the cells of plants affected by several virus diseases. Matz(51) in 1919 described such bodies in mosaic diseases of sugar cane. Kunkel(52) found them in corn; Palm,(53) Goldstein,(54) and Rawlins and Johnson(55) in mosaic-affected tobacco; McKinney, Webb, and Eckerson(56) in wheat rosette, and Smith(57) noted them in potato mosaic. The exact nature of these bodies is little known. Their relation to the cause of mosaic disease has been rightly questioned. Palm designated the bodies found by him in tobacco mosaic *Strongyloplasma iwanowskii.* Nelson(58) in 1922 described certain protozoans in plants affected with mosaic and other related diseases. In the phloëm of the bean and clover plants this author found organisms which he identified as biflagellates. Also organisms were found in the sieve tubes of mosaic tomato and potato leaf-roll plants which he thought to be trypanosomes. Duggar and Karrer(59) soon reported that the structures Nelson described as protozoans were normal inclusions both in healthy and diseased plants and were apparently without significance.

In a recent report on the cytology of mosaic disease Smith(60) confirmed the observations of Goldstein· that the vacuolated bodies in the epidermal and hair cells of leaves of tobacco plants were not associated with the nuclei but were carried through the cells in the protoplasmic streams. This author, however, did not observe any autonomous movements in the vacuolated bodies and in only one instance observed a limiting membrane. Smith observed in *Petunia* two distinct movements of the vacuolated bodies, one a migration through the cell, and the second "a combination of the effect of force exerted on the mobile body by the streaming protoplasm and the apparent changes in form due to its turning over in the streams." Smith also exposed mosaic tobacco juice to the action of the abiotic rays and found that filtered mosaic tobacco juice was inactivated within thirty minutes, while *B. prodigiosus* was killed in thirty seconds.

This she considers as evidence against the theory that the causal agent is an organism. This author concludes that "the vacuolated and granular bodies discussed in this paper are associated directly with the causal agency rather than with the chlorosis which results from the presence of the virus in the plant." Smith favors the view, however, that they do not represent the causal agency, but are rather the product of a reaction between it and the cytoplasm of the cells.

At the present time it may be stated that the opinions of investigators who have worked with the various mosaic diseases is that the protozoan theory of the etiology of mosaic is hardly worthy of serious consideration. As in many animal diseases inclusion bodies in plants have been mistaken for parasites. Just what the nature of these inclusion bodies is must be determined by future investigation. They are not associated with noninfectious chlorosis. This seems to be added evidence that the mosaic diseases are caused by ultramicroscopic viruses, since similar bodies have been found in many other diseases known or thought to be caused by agents of this nature.

BIBLIOGRAPHY

1. BAILEY, Med. Woman's Journ. 34 (1927) 199.
2. SMITH, Phytopath. 5 (1915) 83.
3. ALLARD, Bull. Dept. Agr. No. 40 (1914); Journ. Agr. Res. 3 (1915) 295; 5 (1915) 251; 6 (1917) 649; 7 (1916) 481; 10 (1917) 615; 13 (1918) 619.
4. IWANOWSKI, Beiheft Botan. Centbl. 3 (1893) 266.
5. BEIJERINCK, Centbl. f. Bakt., O. 5 (1899) 27.
6. HEINTZEL, Contagiose Pflanzenkrankheiten ohne Microben, etc., 46 pp., Erlangen. Dissertation (1900).
7. WOODS, U. S. Dept. Agr. Bur. Plant Indus. Bull. 18 (1902).
8. HUNGER, Zeit. Pflanzenkrank. 15 (1905) 257.
9. MAYER, Landw. Vers. Stat. 38 (1886) 450.
10. LODEWIJKS (1910). Abs. in Botan. Centralbl. 114: 518.
11. GILE, Porto Rico Exp. Station Bull. 11 (1911).
12. BACH and CHODAT, Ber. deut. chem. Gesell. 36 (1903) 600.
13. SCHONBEIN, Verhandl. Naturf. Gesell. 1 (1857) 467.
14. LOEW, U. S. Dept. Agr. Rpt. 65 (1900).
15. KASTLE, Pub. Health and Mar. Hosp. Serv. U. S. Hyg. Lab. Bull. 26 (1906).
16. FREIBERG, Ann. Missouri Bot. Gard. 4 (1917) 175.
17. WAKEFIELD, West Ind. Bull. 28 (1921) 197.
18. DUGGAR and KARRER, Ann. Missouri Bot. Gard. 18 (1921) 343.
19. GARDNER and KENDRICK, Ind. Agr. Exp. Sta. Bull. 261 (1922); Bot. Gaz., 73: 469.
20. OLITSKY and NORTHROP, Science 61 (1925) 544.

21. Dickson, Science **62** (1925) 398.
22. Johnson, Wis. Agr. Exp. Sta. Res. Bull. **63** (1925).
23. Schultz, Journ. Agr. Res. **17** (1919) 247.
24. Melchers, Ohio Nat. **13** (1913) 149.
25. Melhus, Phytopath. **7** (1917) 71.
26. Murphy, Agr. Gaz. Canada **4** (1917) 345.
27. Stewart, N. Y. State Agr. Exp. Sta. Bull. **422** (1916).
28. Fernow, N. Y. Agr. Exp. Sta. Mem. **96** (1925).
29. Schultz and Folsom, Journ. Agr. Res. **25** (1923) 43.
30. Blodgett, Phytopath. **17** (1927) 727.
31. Selby, Ann. Rpt. Ohio State Hort. Soc. (1903) 109; Ohio Agr. Exp. Sta. Bull. **214** (1910).
32. Stone, Mass. Agr. Exp. Sta. 22d Ann. Rpt. (1910) 163.
33. Clinton, Conn. Agr. Exp. Sta. Ann. Rpt. (1908) 865; (1916) 430.
34. Gilbert, Phytopath. **6** (1916) 143.
35. Jagger, Phytopath. **6** (1916) 148; **7** (1917) 61; **8** (1918) 32, 74.
36. Doolittle, U. S. Dept. of Agr. Bull. **879** (1920).
37. Elmer, Science **56** (1922) 371.
38. Schultz, Journ. Agr. Res. **22** (1921) 173.
39. Jagger, Journ. Agr. Res. **20** (1921) 737.
40. McClintock and Smith, Journ. Agr. Res. **14** (1918) 1.
41. Brandes, U. S. Dept. Agr. Bull. **829** (1919); Journ. Agr. Res. **19** (1920) 131; **23** (1923) 279.
42. Ledeboer, Arch. Suikerindus. Nederland.-Indië **29** (1921) 1000.
43. Bruner, Rev. Agr. Com. y Trab. (Cuba) **5** (1922) 11.
44. Kunkel, Haw. Planters' Rec. **26** (1922) 58.
45. Edgerton and Taggart, Journ. Agr. Res. **29** (1924) 501.
46. Brandes and Klaphaak, Journ. Agr. Res. **24** (1923) 247.
47. Stevenson, Journ. Dept. Agr. and Labor Porto Rico **4** (1919) 73.
48. Clinton, Conn. Agr. Exp. Sta. Ann. Rept. **38** (1915) 357.
49. Rankin, N. Y. State Agr. Exp. Sta. Bull. **543** (1927).
50. Bennett, Agr. Exp. Sta. Mich. State Coll. Bull. **80** (1927).
51. Matz, Journ. Porto Rico Dept. Agr. **3** (1919) 65.
52. Kunkel, Haw. Sugar Planters' Assoc. Exp. Sta. Bull. **3** (1921) 1.
53. Palm, Deli Proefsta., Medan, Sumatra, Bull. **15** (1922) 1.
54. Goldstein, Bull. Torrey Bot.. Club **51** (1924) 261.
55. Rawlins and Johnson, Am. Journ. Bot. **12** (1925) 19.
56. McKinney, Webb, and Eckerson, Journ. Agr. Res. **26** (1923) 605.
57. Smith, Ann. Bot. **38** (1924) 385.
58. Nelson, Mich. Agr. Exp. Sta. Tech. Bull. **58** (1922) 1–30.
59. Duggar and Karrer, Phytopath. **13** (1923).
60. Smith, Ann. Missouri Bot. Gard. **13** (1926) 425.

ILLUSTRATIONS

Plate 44

Mosaic disease of tobacco.

Plate 45

Fig. 1. Corollas of American varieties of *Nicotiana tabacum*, showing various degrees of mottling produced by the mosaic disease;

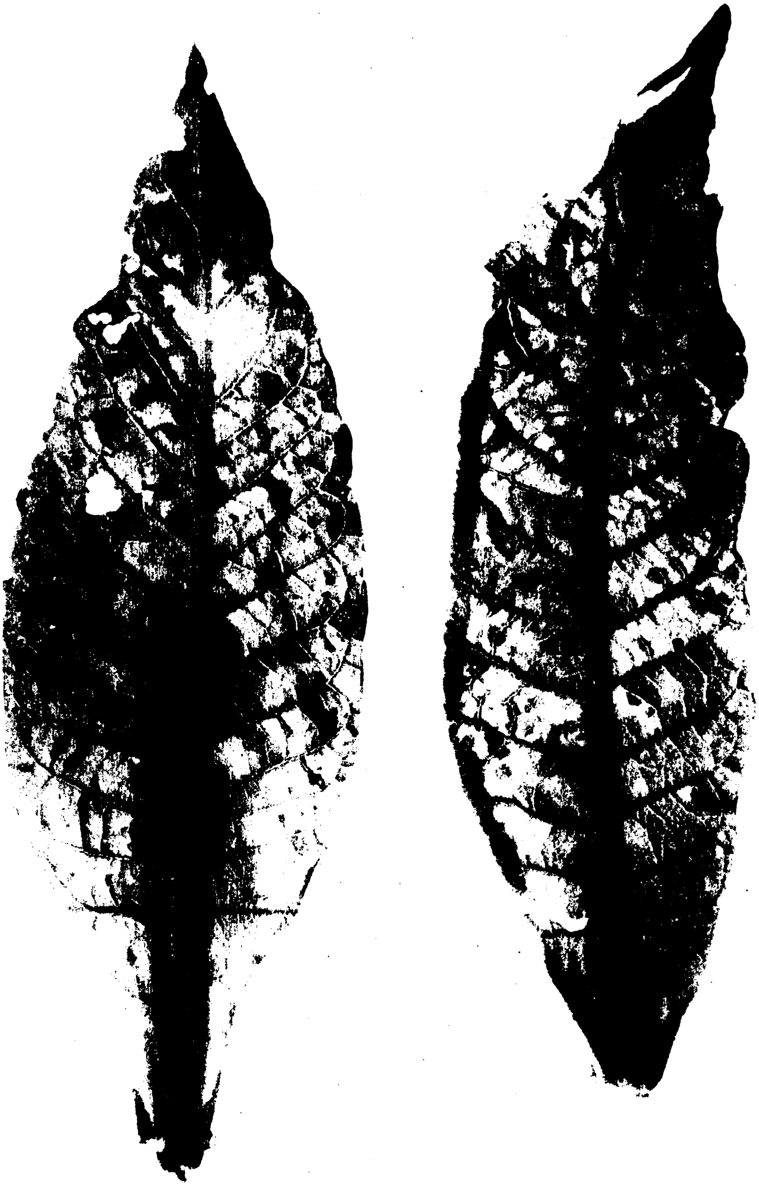

PLATE 44. MOSAIC DISEASE OF TOBACCO.

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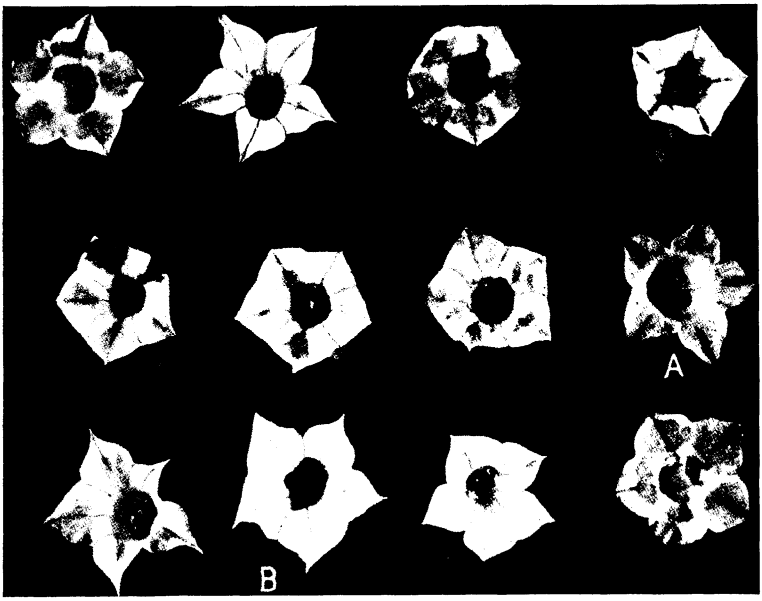

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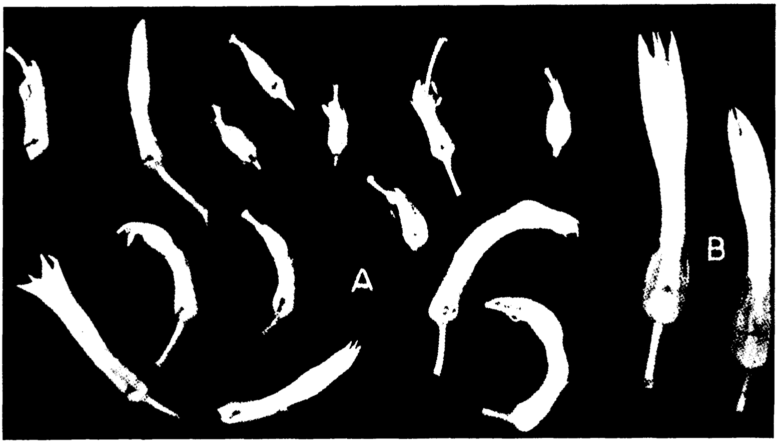

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Fig. 1. Corollas of American varieties of Nicotiana tabacum, showing various degrees of mottling. 2. Buds of Nicotiana tabacum.

PLATE 45. MOSAIC DISEASE OF TOBACCO.

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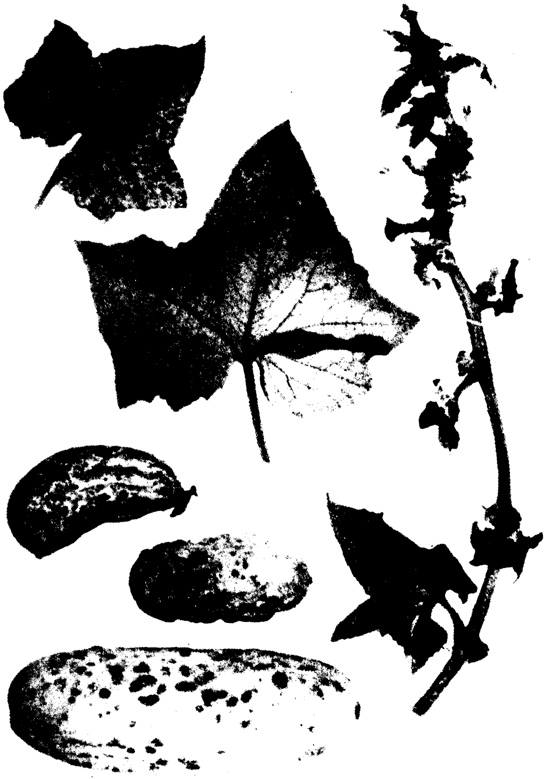

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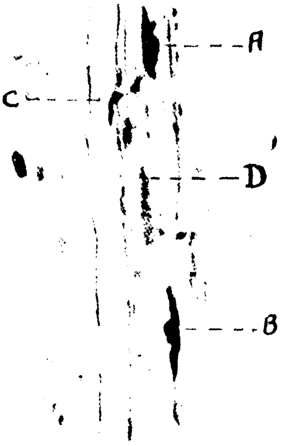

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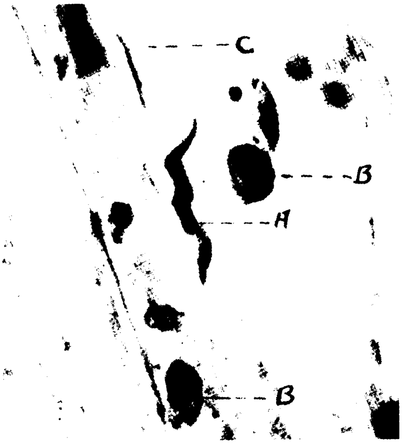

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Fig. 1. Mosaic leaves, fruits, and branch of cucumber. 2 and 3. Mosaic disease in tomato plant.

PLATE 46. MOSAIC DISEASE OF CUCUMBER AND OF TOMATO.

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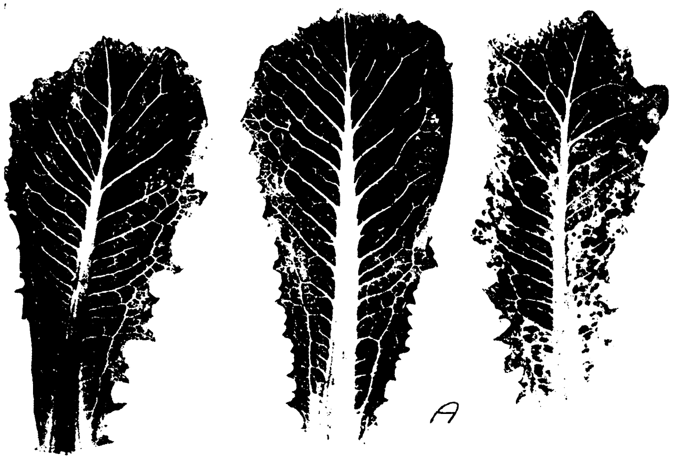

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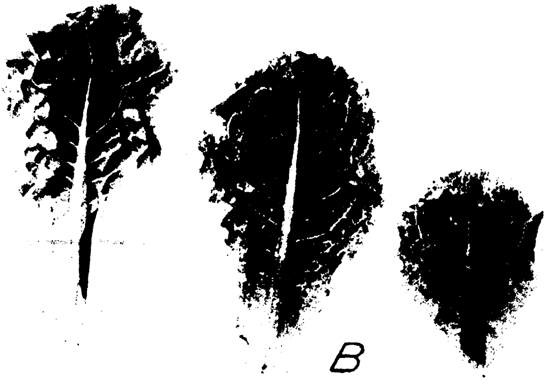

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Fig. 1. Leaves of Romaine lettuce. 2. Young expanding leaves of head lettuce.

PLATE 47.

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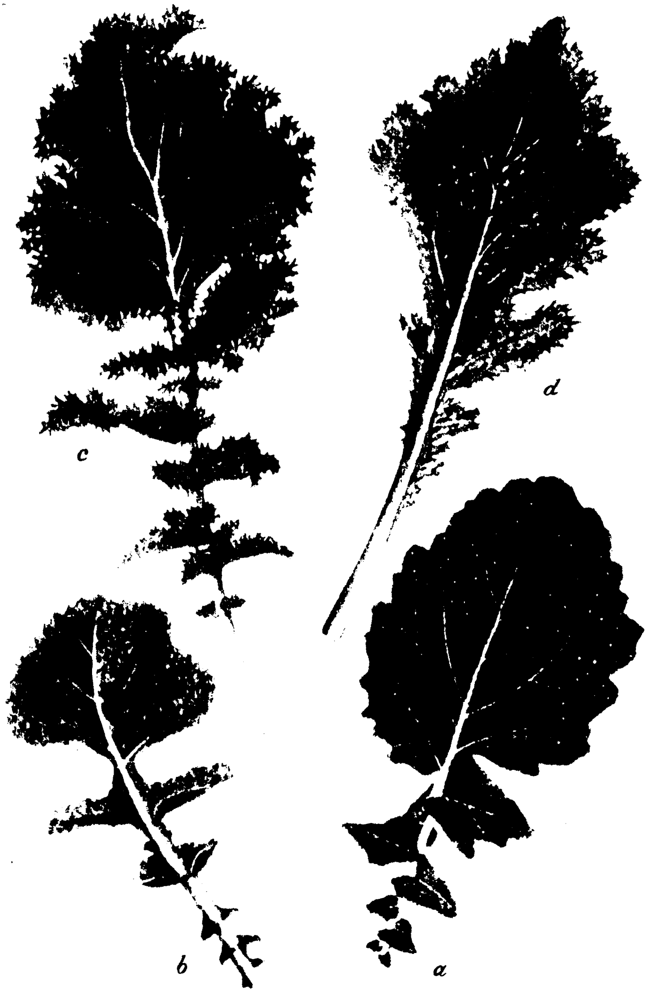

PLATE 48. MOSAIC DISEASE INDUCED BY APHIDS IN TURNIP AND IN MUSTARD.

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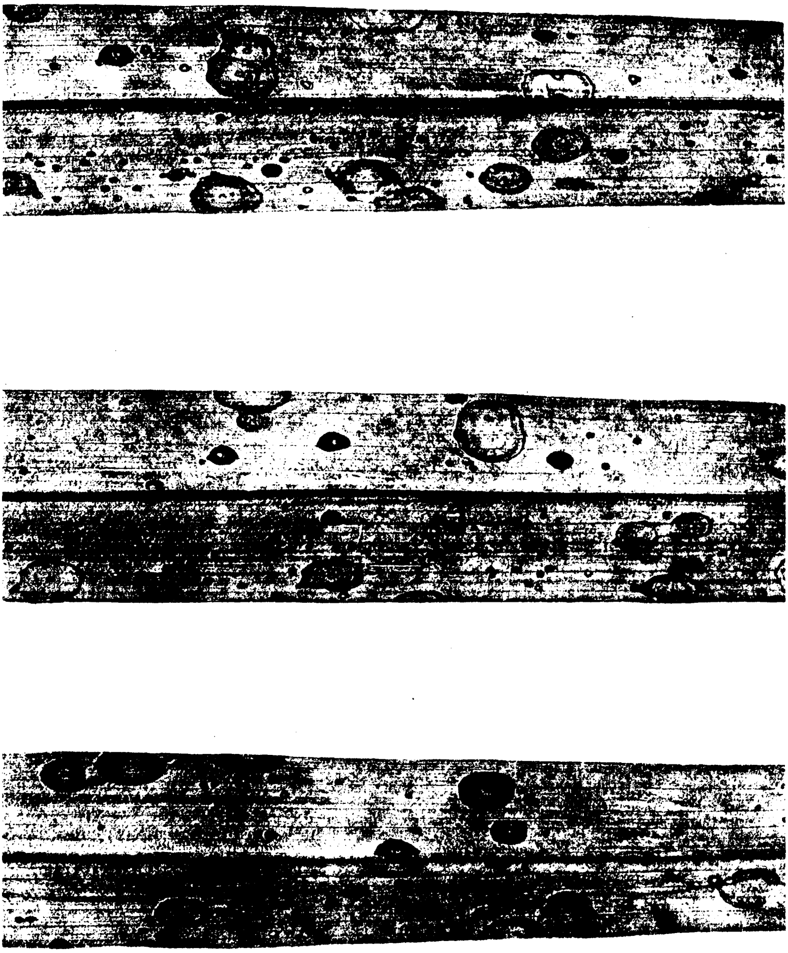

PLATE 49. MOSAIC DISEASE OF SUGAR CANE.

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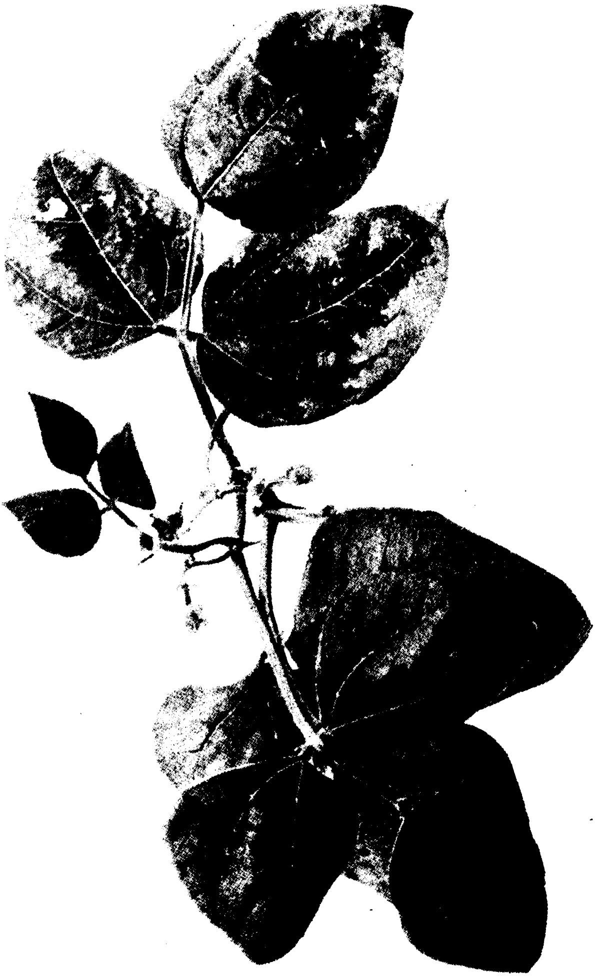

PLATE 50. BEAN PLANT AFFECTED WITH MOSAIC, SHOWING TYPICAL MOTTLING AND DISTORTION OF FOLIAGE.

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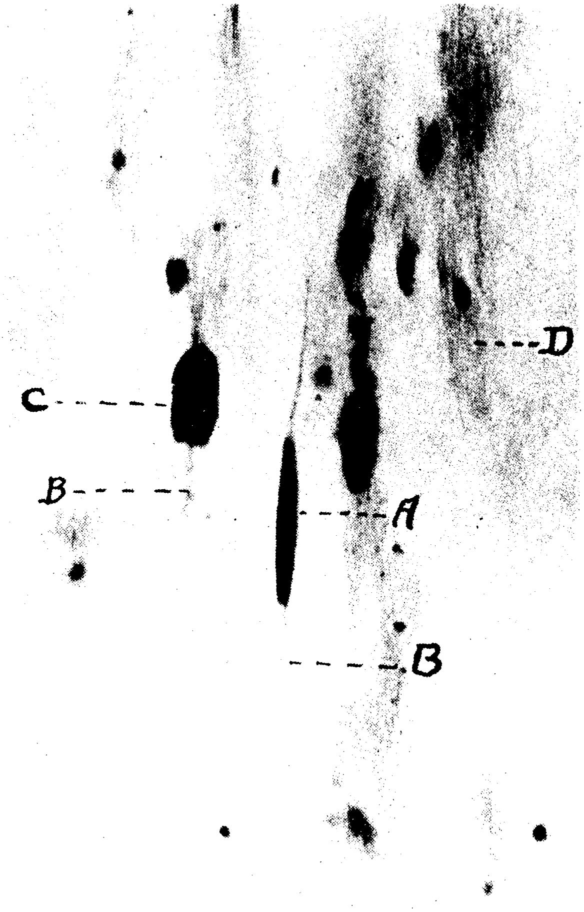

PLATE 51. PHOTOMICROGRAPH OF ELONGATED BIFLAGELLATE PROTOZOAN IN SIEVE TUBE OF MOSAIC BEAN PLANT.

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a, normal pink blossom; *b*, mosaic blossom which is entirely white.

FIG. 2. Buds of *Nicotiana tabacum; a*, showing distorted and other depauperate buds produced by the mosaic disease; *b*, normal, healthy buds. (After Allard.)

PLATE 46

FIG. 1. Mosaic leaves, fruits, and branch of cucumber. (After Doolittle.)

2. Longitudinal section through phloëm tissue of mosaic tomato plant; *a*, two trypanosome-like organisms lying side by side in a sieve tube; *b*, two of the organisms attached to each other by posterior ends of the body; *c*, an organism apparently passing directly through walls of the sieve tube; *d*, sieve tube. (After Nelson.)

3. Longitudinal section of phloëm tissue in tomato plant affected with mosaic; *a*, trypanosome; *b*, cell nucleus; *c*, sieve tube. (After Nelson.)

PLATE 47

FIG. 1. Leaves of Romaine lettuce. Leaf in center from healthy plant; two others from mosaic plants, one showing pronounced type of mottling and the other general yellowish discoloration.

2. Young expanding leaves of head lettuce. Leaf on left from healthy plant; two others from plant in early stages of the mosaic disease. (After Jagger.)

PLATE 48

Mosaic disease; *a*, leaf from healthy turnip, control to leaf, *b*, from mosaic turnip, mosaic induced by aphids; *c*, leaf from healthy mustard, control to leaf, *d*, from mosaic mustard, mosaic induced by aphids. (After Schultz.)

PLATE 49

Mosaic disease of sugar cane.

PLATE 50

Bean plant affected with mosaic, showing typical mottling and distortion of foliage. (After Nelson.)

PLATE 51

Photomicrograph of elongated biflagellate protozoan in sieve tube of mosaic bean plant; *a*, elongated organism of type 1; *b*, flagellum; *c*, ovaliform flagellate, type 2; *d*, cell nucleus with deeply-stained nucleolus in center. (After Nelson.)

CHAPTER XV

THE BACTERIOPHAGE

Definition.—The word bacteriophage means "bacteria eater" and represents the term applied to a bacterial lytic principle by d'Herelle(1) in 1917. At present the bacteriophage can only be defined as a "substance," "agent," or "principle" which is filterable through the finest porcelain filters and which is capable of bringing about the dissolution of certain bacteria. Some investigators regard the bacteriophage as a living filterable virus; others believe the lytic principle to be of the nature of an enzyme; while there are those who think of this "agent" as a product of bacterial dissociation, autolysis, or as a hereditary by-product of the bacteria. Since opinion has been so divided in the past decade during which this agent has been studied, it is impossible to define its nature accurately. Of its properties and activities we know more. At the end of this section will be found a critical opinion of its nature based upon the facts that have been adduced by accepted experimental methods. For the present we will consider the bacteriophage as a filterable lytic agent, active in extremely high dilutions, capable of increasing in quantity at the expense of the lysed bacterium, and as a "substance" almost constantly present in the intestines of man and animals.

History and distribution.—In 1915 Twort(2) published the paper "An investigation on the nature of ultramicroscopic viruses." In this paper Twort described certain transparent areas in a culture of staphylococci in which no cocci grew. Touching one of these transparent areas with a sterile platinum loop and then drawing the loop across the surface of a twenty-four-hour agar culture of staphylococci, he found, after a few hours, marking the tract of the loop a streak which had become clear and transparent. Filtering his material from these transparent areas through a Berkefeld filter, he found that the filtrate would dissolve and kill most of the organisms in fresh staphylococcus cultures even in dilutions of one to a million.

It is interesting to note that this first scientific description of a "lytic principle" for bacteria, that is transmissible in series, was discovered in a culture of an organism that is Gram-positive. Except for the observation of Hankin in 1896 this is the first printed record concerning the bacteriophage. Hankin,(3) nearly twenty years before, described the bactericidal action of the water of the Ganges and Jumna Rivers in India for the cholera vibrio, but the idea of bacteriophage was not suggested by him. To Twort belongs the credit for being the first to bring this phenomenon to the attention of other investigators.

Previous to 1916 d'Herelle was interested in a peculiar disease affecting locusts that he believed to be caused by a filterable virus, although coccobacillus was easily cultivated from the infected locusts and frequently presented cultural irregularities which puzzled him. Some colonies of this bacillus possessed indented irregular contours and at times there were areas entirely free from growth. D'Herelle was inclined to consider this disease as caused by a filterable virus but having an "associated" organism, the coccobacillus, such as exists in hog cholera. Experiments with this disease of locusts led to his observations of the bacteriophage. In one of his books on the bacteriophage d'Herelle describes his first experiments which led to the discovery of the bacteriophage in the stools of dysentery patients. He says:

> In August, 1916, an adult with a severe bacillary dysentery (Shiga) was under treatment in the Pasteur Hospital. Each day about 10 drops of the stool were collected and placed in a tube of bouillon. After incubation over night the suspension was filtered through a Chamberland candle. Into some bouillon, previously inoculated with Shiga bacilli, about 10 drops of this filtrate were placed, and the material was returned to the incubator at 37° C. . . . Throughout the duration of the disease, all of the tubes, prepared each day in the same manner, gave normal cultures of B. dysenteriae. One day, the tube prepared the day before remained sterile. Investigation showed that the patient gave evidence of notable improvement, and, as appeared later, this was shortly followed by definite convalescence. . . . To the bouillon thus inoculated and containing filtrate, and which had remained to all appearances sterile, a suspension of Shiga bacilli derived from a fresh agar culture was added to yield a marked turbidity. This tube was placed in the incubator. After about ten hours it was again clear. . . . This, of course, made it at once apparent that my first hypothesis was of necessity false, the truth of the matter being that the fecal material used in preparing the filtrate contained something which dissolved the dysentery bacilli. Nevertheless, my

first hypothesis had one virtue, since, as it had led me for such a long time to consider the question of a virus pathogenic for the man or the animal, it offered the suggestion that the dissolving principle might be a virus pathogenic for the bacterium.

Thus the discovery of the bacteriophage was made by d'Herelle in the stools of dysentery patients, and the filterable virus theory of the nature of the bacteriophage was conceived. During the past ten years d'Herelle has attempted to prove his theory of the virus nature of the bacteriophage. Other investigators have presented experimental data opposing this view, which to the conservative mind has appeared fantastic and a conclusion unwarranted by the facts that have been presented in its favor.

That there exist diverse strains of bacteriophage is now well recognized. Sewage universally contains bacteriophage. Consequently rivers receiving sewage from towns and cities frequently contain different strains of bacteriophage. We have isolated such strains from various streams and sewage supplies in America, Porto Rico, and the Philippines. Isolation of bacteriophage active against the colon bacillus is a very simple matter with sewage as a source of material. Strains of bacteriophage have also been isolated from the stools of man and animals, from the urine and blood of patients, from old laboratory cultures, from the nodules of plants, from the tissues of man and animals, and from the intestines of silkworms. From the Pasig River in the Philippines we have isolated bacteriophage active against *B. coli, B. typhosus, B. dysenteriæ* Shiga, and *B. dysenteriæ* Flexner. In this stream untold numbers of carabaos wallow during every hour of the day. Excreta from such animals have been found by Basaca to be loaded with bacteriophage. Bacteriophage has been isolated from the water of the Seine by Dumas,(4) and Collins(5) has reported its presence in the Huron River in Michigan. It can be stated definitely that all streams receiving sewage will be found to contain bacteriophage lytic for some organism, practically always for some strain of *B. coli.* The bacteriophage then originates in the intestines of man and animals and perhaps other forms of life such as insects, etc. Since the intestinal tract of man and animals is universally contaminated with *B. coli,* a commensal microörganism, it is suggested that the bacteriophage is in many instances associated with this bacterium or some other bacterial form. Experimental evidence to support this suggestion will be presented later.

Theories concerning the nature of the bacteriophage.—Several theories or hypotheses have been suggested to explain the phenomenon of bacteriophagy. D'Herelle, since his first work with the lytic principle, has considered the bacteriophage as a living ultramicroscopic and filterable virus, foreign and parasitic to bacteria. Kabeshima(6) has suggested that the bacteriophage is a chemical principle foreign to the bacterium. This author suggests the possibility of a catalytic substance in the intestinal tract of animals which brings about the dissolution of bacteria by activating some proferment present in the bacteria. Bordet and Cuica(7) suggested the idea that bacteria undergo a nutritive vitiation under the influence of some product manufactured by leucocytes, and further that this vitiation is hereditary since the phenomenon is transmissible indefinitely in series. D'Herelle speaks of this concept as the hypothesis of an "abnormal inert principle." Kuttner(8) explains the nutritive vitiation of the bacterium upon the basis of some ferment present in the intestinal tract of animals. A similar idea to that of Bordet and Cuica has been suggested in one of my own publications on this subject.(9) Lisbonne and Carrére(10) have suggested the theory that the bacteriophage is the result of a bacterial antagonism. Seiffert(11) has suggested an exogenous autolysis as the cause of the phenomenon. Doerr(12) thinks of the bacteriophage as a toxin that affects the bacterial metabolism. This author would place other agents such as the viruses of rabies, vaccinia, encephalitis lethargica, and sarcoma in this category. A large number of investigators favor the idea that the bacteriophage is simply a normal autolysis. D'Herelle replies to the proponents of this theory that it is strange that the bacteriophage phenomenon occurs with young bacteria having no natural autolytic tendency, and does not take place with old bacteria which autolyse spontaneously. Bail(13) believes that the lytic principle is normally present in the bacteria and is a living substance. There is also the possibility that the lytic principle may be abnormal to the cell, yet living and derived from the bacteria.

There are many hypotheses concerning the nature of the bacteriophage, and in fairness one must state that d'Herelle has presented a theory of the nature of bacteriophage that no one has been able to refute completely or by experimental evidence prove wrong. Arguments based upon careful experimental work have been presented for most of the opposing theories, but no single clear-cut experiment has been performed that will prove any one of these hypotheses and disprove d'Herelle's idea of the living filterable virus nature of the lytic principle. We do not advocate the virus theory of the bacteriophage, because we feel that the experimental evidence so far advanced does not prove its living nature, but in justice to d'Herelle it must be admitted that he has presented a thesis that is almost convincing and exceedingly difficult to discredit. For a detailed discussion and analysis of the various hypotheses that have been advanced to explain the phenomenon of bacteriophagy, the reader is referred to d'Herelle's books on the subject.

Bacteria for which lytic principles have been described.—A wide variety of bacteria has been found susceptible to the phenomenon of bacteriophagy. The list includes *Bacillus dysenteriæ* Shiga, His, and Flexner; *B. gallinarum; Pasteurella bovis; B. pestis; B. typhosus,* para A and B; *B. suipestifer; B. enteritidis; B. typhi-murium; B. coli;* the Friedländer bacillus; the bacillus of flacherie; *B. proteus;* the bacillus of swine fever; *B. diphtheriæ;* nodule bacteria of Leguminosæ; *B. subtilis; Vibrio choleræ; Staphylococcus gratia; Enterococcus; Streptococcus; B. pyocyaneus;* pseudobacteriophage for *B. anthracis;* thermophilic bacillus T 60; and psychrophilic bacteria.

Properties of the bacteriophage.—It is well recognized that there exist many different "strains" of bacteriophage. It has also been well established that all "strains" of bacteriophage are filterable through ultrafilters. It has further been demonstrated by d'Herelle, Bronfenbrenner and Korb,(14) McKinley and Holden,(15) and others that the lytic principle is particulate. According to d'Herelle these particles are about the size of the micella of serum globulin. Prausnitz(16) states that the diameter of the bacteriophage particle is about the size of the micella of collargol used in his experiments, or about 20 millimicrons. Von Angerer(17) gives a figure of 30 millimicrons for its diameter, Jötten(18) and Arnold(19) have described experiments which demonstrate beyond doubt that the bacteriophage is diffusible through the agar upon which is planted the bacteriophage with its susceptible microörganism. The bacteriophage is evidentally nonvolatile, although it may be carried over in distillates by droplets if care is not taken to prevent this. By titration it has been shown by d'Herelle and others that particles of bacteriophage tend to form a sediment on the bottom of a vessel on standing and to some degree by centrifugation. That the particles are certainly unevenly distributed in a liquid sus-

TABLE 11.—*Number of lytic plaques on plates of every 0.1 cubic centimeter of each progressive dilution (1 cubic centimeter) of bacteriophage.*

[+, large lytic areas, no plaques; isolated colonies of *B. coli* "D;" W, washings from dilution tube and pipette; *, mixture of plaques and large lytic areas; accurate count not possible.]

Dilution of bacteriophage.	Plates.									
	1	2	3	4	5	6	7	8	9	10
Concentrated	+	+	+	+	+	+	+	+	+	+W
1:10	+	+	+	+	+	+	+	+	+	+W
1:100	+	+	+	+	+	+	+	+	+	+W
1:1,000	+	+	+	+	+	+	+	+	+	+W
1:10,000	*20	479	467	356	475	367	357	274	50W	90W
1:100,000	55	60	70	67	58	60	81	13	20W	24W
1:1,000,000	12	11	7	8	13	4	9	3	0W	1W
1:10,000,000	0	7	0	0	0	3	4	1	1	0W
REPETITION WITH SECOND 1 CUBIC CENTIMETER OF LYTIC PRINCIPLE.										
Concentrated	+	+	+	+	+	+	+	+	+	+W
1:10	+	+	+	+	+	+	+	+	+	+W
1:100	+	+	+	+	+	+	+	+	+	+W
1:1,000	+	+	+	+	+	+	+	+	+	562W
1:10,000	677	631	597	652	671	493	587	593	82W	111W
1:100,000	93	74	91	90	76	95	86	52	7W	11W
1:1,000,000	12	13	6	11	4	10	4	8	0W	2W
1:10,000,000	2	1	0	1	0	1	1	0	0W	2W

pension has been indicated in our work. The bacteriophage is not soluble and exists only in suspension, perhaps in a colloidal state. Wollman(20) thought that he was able to digest the lytic principle with trypsin, but further work indicated that this destruction is only partial. The possibility exists that in bouillon the bacteriophage particle is adsorbed on to protein molecules. This is indicated by the fact that the precipitate formed from bouillon by the addition of acetone or alcohol is found to contain the lytic principle in a very high concentration. D'Herelle states that one strain of bacteriophage has remained alive in a sealed ampule for nine years. He says: "When first prepared there were over 2,000 million corpuscles per cubic centimeter, after four years the number was reduced to only 100 millions, after nine years to but 40 millions." The bacteriophage "multiplies" in an alkaline medium, though a few "strains" have been described which "multiply" in an acid medium. In general the bacteriophage particles floculate under the influence of acids (Da Costa Cruz), a phenomenon that also occurs with the majority of bacteria. Since these bacteria carry a negative electric charge it follows that the bacteriophage particle is also charged negatively. D'Herelle terms it a "negative colloid." The bacteriophage is also precipitated by saturation with ammonium sulphate according to Maisin,(21) but it may be recovered still virulent from the precipitate as in the case of an acetone or alcohol precipitate. Magnesium apparently acts in the same way according to de Poorter and Maisin.(22) In acetone or alcohol precipitates the bacteriophage is destroyed if contact is prolonged for several days.

The bacteriophage particle is adsorbed by a number of substances. The protein in bouillon has already been referred to. Various authors have demonstrated that the bacteriophage is adsorbed by infusorial earth and kaolin (Seiffert,(23) Gildemeister and Herzberg,(24) and others). Much of the work done on adsorption, however, has not taken into account the hydrogen ion concentration of bacteriophage suspension, and opinion is at variance upon this property of the bacteriophage. D'Herelle believes there is relatively little adsorption of bacteriophage by these substances.

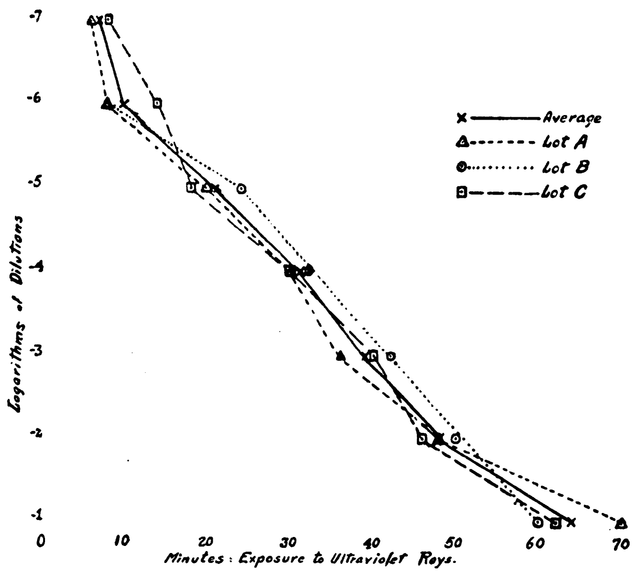


FIG. 7. The minimal lethal exposures (abscissa) for bacteriophage in dilutions 1 : 10 to 1: 10,000,000 in three broths, A, B, and C, plotted according to the logarithms of the dilutions (ordinate).

The bacteriophage is also affected by irradiation. Appelmans(25) has inactivated bacteriophage by exposure to ultra-violet rays for ten minutes. Gildemeister(26) states that the bacteriophage is sensitive to ultra-violet light as are bacteria. Gerretsen, Gryns, Sack, and Söhngen(27) found that ultra-violet light destroyed *B. radicicola* but not its lytic principle after exposure for fifteen minutes. McKinley, Fisher, and Holden,(28) however, have found that a strain of bacteriophage lytic for *B. coli* is destroyed by ultra-violet light much in the same way as are two strains of known filterable viruses; namely, herpes and Levaditi's so-called encephalitis virus. Exposure to ultra-violet light at a distance of one foot for forty minutes is sufficient to attenuate or destroy both the bacteriophage and the two filterable viruses employed. *Bacillus coli* was not destroyed by this exposure. Later experiments by these authors showed that much shorter periods of exposure were effective in destroying both the lytic principle and the filterable viruses mentioned. Fisher and McKinley(29) exposed various concentrations of bacteriophage to the action of ultra-violet light and concluded that the resistance to ultra-violet rays of the lytic principle is directly proportional to its concentration and appears to be a logarithmic function thereof. The effect produced by ultra-violet light is not a photosensitization to heat. Brutsaert (30) found that radium emanations did not destroy the bacteriophage.

As has been mentioned there are many different "strains" of the bacteriophage. This is indicated by several properties that they possess. Some strains of bacteriophage always produce very minute pinhead plaques on solid cultures, while others produce plaques many times as large. To some extent the size of the plaques may be controlled by increasing the concentration of the agar. This has been demonstrated by Bronfenbrenner and Korb. However, on a uniform agar concentration different "strains" of lytic principle will exhibit different-sized plaques. Differences between "strains" of bacterophage are also noted in their response to heat. One "strain" may be destroyed at a temperature of 65° C., while another will require 70 or 75° C. when heated for thirty minutes. D'Herelle states that heat destruction is preceded by attenuation and that 75° C. may, in general, be regarded as the thermal death point of the bacteriophage. Bacteria sensitive to the action of such a lytic principle are of course destroyed at much lower temperatures. The multiplicity of bacteriophage "strains" is also indicated by the specificity of the various strains. Within certain limits adaptation of the bacteriophage to more than one organism is possible but not always. Some "strains" seem to be markedly specific.

The bacteriophage is susceptible to the action of various chemicals and disinfectants. The response of the bacteriophage to these substances depends somewhat upon the race; various races respond differently. In general it may be said that in its resistance to chemicals and disinfectants the bacteriophage is comparable to the spores of bacteria. In some instances it appears to be more resistant than spores to the disinfectant employed. This resistance also depends somewhat upon the temperature at which the destructive agent is permitted to act. For example, Bronfenbrenner and Korb found that the bacteriophage is rapidly destroyed by absolute alcohol at ordinary temperatures, but that it resists absolute alcohol for five or six days if kept at 0° C.

Facts that favor the living nature of the bateriophage.—The fact that the bacteriophage increases in quantity at the expense of the lysed bacterium is the fundamental argument upon which d'Herelle bases his theory of the virus nature of the bacteriophage. This he regards as evidence of reproduction and multiplication. The theory of the living nature of the bacteriophage is also supported by the fact that, within certain limits, the bacteriophage possesses the power of adaptation and assimilation. These are regarded as the combination of characters that constitute the criterion of life.

Opposing view.—Many investigators have been unwilling to accept these criteria as proved in the case of the bacteriophage. Increase in quantity of bacteria at the expense of the lysed bacterium is not regarded in the minds of many as representing reproduction in the sense in which this word is usually employed. It is argued for instance that the bacteriophage may represent merely a lytic principle elaborated by the bacterium itself and be enzymatic in nature. Bordet believes that the bacterium is vitiated by the action of the cells of the host and that this vitiation continues through the subsequent generations of the strains. This is suggested in the strain of *B. coli* of Lisbonne and Carrère which manufactures a lytic principle active against a strain of *B. dysenteriæ* Shiga. D'Herelle regards this strain of *B. coli* as contaminated with the bacteriophage. Such a strain, however, it has been shown by us, produces antilysins for the lytic principle when injected into normal rabbits when primary cultures of this organism in broth show no evidence of the phenomenon of bacteriophagy. D'Herelle will refute this with the statement that the bacteriophage contaminant is not present in sufficient concentration in the primary culture to manifest itself but even in its weak concentration it is antigenic. This is, of course, possible; however, it is not probable. *Bacillus coli* Lisbonne in culture presents no evidence that it possesses the power of elaborating a lytic principle for *B. dysenteriæ* Shiga. In other words *B. coli* Lisbonne itself is not affected by the lytic principle. Therefore, the lytic principle active against *B. dysenteriae* Shiga is not increasing in quantity (in the d'Herelle sense) when it exists in a pure culture of *B. coli* Lisbonne for the simple reason that *B. coli* Lisbonne is not sensitive to it. In hundreds of subcultures of *B. coli* Lisbonne we have never seen any evidence of lytic plaques or any other indication that a bacteriophage was present in the culture. The bacteriophage must, according to d'Herelle, increase in quantity at the expense of the sensitive bacterium. In the *B. coli* Lisbonne culture the sensitive bacterium so necessary for the "multiplication" of the lytic principle is missing; and further it is found that subcultures generation after generation of *B. coli* Lisbonne possess the power of elborating bacteriophage for *B. dysenteriæ* Shiga.

If we accept d'Herelle's contention that *B. coli* Lisbonne is contaminated with the bacteriophage and knowing that the bacteriophage does not lyse *B. coli* Lisbonne and hence, in d'Herelle's sense in unable to "multiply" at the expense of this organism, how then are we to explain the fact that each generation of *B. coli* Lisbonne is capable of elaborating the lytic principle for *B. dysenteriæ* Shiga? *Bacillus coli* Lisbonne divides approximately every seventeen minutes. If each bacterium contains bacteriophage or possesses bacteriophage adsorbed upon its surface, this quantity of lytic principle will be divided with each division of the microbe. One hesitates to attempt to calculate the dilution of lytic principle which would take place after several subcultures of this organism. It is known, however, that a subculture prepared from one colony of *B. coli* Lisbonne, after several generations, is still capable of elaborating lytic principle active against *B. dysenteriæ* Shiga. This is strong evidence against the assumption that *B. coli* Lisbonne is "contaminated" with the bacteriophage and to our mind indicates definitely that

B. coli Lisbonne possesses the inherent ability of elaborating lytic principle active for *B. dysenteriæ* Shiga. In other words, this property is functional and is perhaps a metabolic process upon the part of *B. coli* Lisbonne. This is strong indication that the lytic principle is an enzyme elaborated by *B. coli* Lisbonne in this particular instance.

While many investigators have been willing to accept the hypothesis of d'Herelle that the bacteriophage is a living ultramicroscopic virus there are still those who are not willing to accept this view as final. Though d'Herelle's view offers much in its favor the evidence is not complete. For the present we prefer to consider the bacteriophage as an ultramicroscopic particulate substance, diastatic in its action, granular in form, inanimate, and probably derived from the bacterial cell during a stage in its life cycle as a result of the influence of tissue cells of the host upon the bacterium. We have likened the bacteriophage to the zymogenic granules of the pancreas and its lytic action to that analogous to the action of zymogen, and enzyme elaborated by the zymogenic granules. The response of the lytic principle to external agents such as heat, chemicals, disinfectants, and ultra-violet light may be considered analogous to that of various enzymes. The bacteriophage granules, which are precursors of the lytic principle, are elaborated by the bacterial cell, a phenomenon brought about by the cells of the host acting upon the bacterium.

Some other criterion is essential to establish the living nature of the bacteriophage. Bronfenbrenner(31) has been unable to detect respiration, and we have reported similar conclusions.(32) If this could be demonstrated it would be the strongest argument in favor of the living nature of the lytic principle.

Hadley(33) suggests a joint relation of microbic dissociation and the phenomenon of the bacterophage. He states:

> I therefore regard the suggestion justifiable that active microbic dissociation and the phenomenon of the bacteriophage may represent merely two different stages in a single phase of normal reproductive and physiologic behavior which exists for a purpose presumably adaptive.

This author ventures the further speculation that it may eventually be demonstrated, not that a foreign filterable virus gives rise to dissociation and to autolysis in the d'Herelle sense; but, on the contrary, that the fundamental physiologic reaction, of which both microbic dissociation and transmissible autolysis are only different modes of expression, gives rise to the filterable virus. In a more recent publication Hadley(34) states:

> The working hypothesis . . . which I have accepted, is that the bacteriophage is either a definite stage in the cyclogeny of the bacterial species, or a functionally active particle accessory to one of these stages; and by the term "accessory" I mean possessing complementary or reciprocal biologic significance, such, for example, as the relation of sperm cell to ovum. With such a conception there is not any priority of significance in the relation between bacteriophagic corpuscle and the cell that generates it; or, indeed, between the bacteriophagic corpuscle and the cell which it "attacks." Both elements are necessary components of a definite reproductive mechanism possessed by many, if not by all, bacteria. This constitutes the nucleus of what I have termed my "homogamic theory" of bacteriophage action.

The reader is strongly urged to study the work of Hadley which has been cited above. Of the hundreds of articles that deal with the bacteriophage those of Hadley have given us the most logical and critical analysis of this phenomenon. It is unfortunate that a review of this kind does not permit a detailed discussion of his work and views on this subject.

BIBLIOGRAPHY

1. D'HERELLE, The Bacteriophage (translation). Williams and Wilkins Co., Baltimore (1922); Immunity in Natural Infectious Disease. Williams and Wilkins Co., Baltimore (1924); The Bacteriophage and its Behavior. Williams and Wilkins Co., Baltimore (1926).
2. TWORT, Lancet **2** (1915) 124; Brit. Journ. Exp. Path. **1** (1920) 237; Brit. Med. Journ. **2** (1922) 293; Journ. State Med. **31** (1923) 351.
3. HANKIN, Ann. Inst. Pasteur **10** (1896) 511.
4. DUMAS, Compt. rend. Soc. de biol. **83** (1920) 1314.
5. COLLINS, Thesis, University of Michigan (1924).
6. KABESHAMA, Compt. rend. Soc. de biol. **83** (1920) 219; 471.
7. BORDET and CUICA, Compt. rend. Soc. de biol. **83** (1920) 1293; (1920) 1296.
8. KUTTNER, Proc. Soc. Exp. Biol. and Med. **18** (1921) 222.
9. MCKINLEY, Journ. Lab. and Clin. Med. **9** (1923) No. 3.
10. LISBONNE and CARRÉRE, Compt. rend. Soc. de biol. **86** (1922) 569.
11. SEIFFER, Zeitschr. f. Hyg. u. Infektionskrankh. **98** (1922) 482.
12. DOERR, Klin. Wchnschr. **1** (1922) 1489; 1537.
13. BAIL, Bull. techn. d. Sc. méd. **1** (1925) 23.
14. BRONFENBRENNER and KORB, Journ. Exp. Med. **42** (1925) 483.
15. MCKINLEY and HOLDEN, Journ. Infect. Dis. **39** (1926) 451.
16. PRAUSNITZ, Klin. Wchnschr. **1** (1922) 1639.
17. VON ANGERER, Arch. f. Hyg. **92** (1924) 312.
18. JÖTTEN, Klin. Wchnschr. **1** (1922) 2181.
19. ARNOLD, Journ. Lab. and Clin. Med. **8** (1923) 720.

20. WOLLMAN, Compt. rend. Soc. de biol. **90** (1924) 59; **92** (1925) 552.
21. MAISIN, Compt. rend. Soc. de biol. **84** (1921) 468.
22. DE POORTER and MAISIN, Arch. internat. pharmacod. **25** (1921) 473.
23. SEIFFERT, Klin. Wchnschr. **2** (1923) 1479.
24. GILDEMEISTER and HERZBERG, Klin. Wchnschr. **3** (1924) 186.
25. APPLEMANS, Compt. rend. Soc. de biol. **86** (1922) 508.
26. GILDEMEISTER, Centralbl. f. Bakt. I. Orig. **89** (1922) 181.
27. GERRETSEN, GRYNS, SACK, and SÖHNGEN, Centralbl. f. Bakt. **2** (1923–24) Orig. 60, 311.
28. MCKINLEY, FISHER, and HOLDEN, Proc. Exp. Biol. and Med. **23** (1926) 408.
29. FISHER and MCKINLEY, Journ. Infect. Dis. **40** (1927) 399.
30. BRUTSAERT, Compt. rend. Soc. de biol. **89** (1923) 90.
31. BRONFENBRENNER and REICHERT, Proc. Exp. Biol. and Med. **24** (1926) 176.
32. MCKINLEY and COULTER, Proc. Exp. Biol. and Med. **24** (1927) 685.
33. HADLEY, Journ. Infect. Dis. **40** (1927) 1–312.
34. HADLEY, Journ. Infect. Dis. **42** (1928) 261–434.

CHAPTER XVI

FILTERABLE FORMS OF BACTERIA, YEASTS, AND SPIROCHÆTES

It is well recognized that certain bacteria and spirochætes under favorable conditions will pass through the pores of various types of filters. This is particularly true with certain grades of filters if pressure is employed. Examples such as the bacillus of guinea-pig pneumonia which passes the Berkefeld V filter, a spirillum isolated by von Esmarch which passes through Berkefeld and Chamberland F filters, Borrell's water flagellate which passes through coarse filters, spirochætes which pass Berkefeld N, V, and W filters acording to Wolbach and Binger, Novy, and others, are frequently quoted in text books.

In addition to the above forms various other bacteria are apparently filterable through filters under certain conditions. Filterable forms of *B. tuberculosis, B. dysenteriæ, B. typhossus* vibrio, *B. coli, M. aureus,* streptococci, *B. proteus,* yeasts, etc., have been described.

The question of filtration has been mentioned in Chapter I. We have seen that there are many factors involved and that filtration is not a sharp dividing line based upon the size of the particle to be filtered. It is largely a matter of gradation but, like diffusibility, is only relative. While we have learned a great deal about filtration there are many aspects of the subject that are not fully understood.

Filtration may be performed with a variety of filters made with many different substances. Porcelain, infusorial earth, asbestos, silica jelly or gelatin, collodion, paper pulp, calcium carbonate and magnesium oxide, plaster of Paris, animal charcoal, animal membranes in vitro and in vivo, have all been employed. Of all these types of filters the ultrafilters prepared as sacks or membranes with collodion have apparently offered more advantage with regard to the determination of size of various particles. Even with such filters, however, only the approximate size of particles has been determined by comparing one substance with another with regard to its filtration qualities.

It is well known that any two filters made of the same material, by the same process, and under identical conditions may vary one from the other in certain respects. Furthermore, a filter once used is theoretically no longer the same filter it was in the beginning. Regardless of the type of filter employed the actual operation of filtration involves the same principles. Pressure, suction or gravity, is employed as the controlling force. This is very important, for a substance that ordinarily is non-filterable without pressure may prove filterable through a given filter if a certain pressure is employed. With regard to spirochætes, for example, it has been thought that the flexibility of the microbe itself may permit its passage through the filter, particularly when pressure is employed. In other words such an organism may be forced or sucked through the pores of a filter. This may be regarded as a pure mechanical process. Furthermore, it has been demonstrated that certain organisms may in time grow through the pores of a filter without pressure from either side of the filter aiding its passage. Bronfenbrenner and Muckenfuss(1) have shown that as a filter is employed in a given operation it is found impermeable to bacteria early in the course of filtration but later, after prolonged filtration, bacteria easily pass through its pores and can be cultivated from the filtrate. This is apparently due to a coating of the filter with albuminous material and an alteration of its electric charge.

It will be recalled from Chapter I that Kramer(2) has shown that by preparing a filter of calcium carbonate and magnesium oxide of positive electrical charge bacteria, viruses, and colloids used in his experiments may be withheld though these agents readily pass through filters made of siliceous material carrying a negative charge.

Various factors then should be taken into account in connection with the subject of filtration. Length of time of filtration, type of filtering, reaction of the filter and of the material to be filtered, the dilution of the material to be filtered, type and amount of force applied against the filter, the electrical charge of the filter and of the particles to be filtered, the material used for control work, the amount of solid matter present in the material to be filtered, its viscosity, filtration technic, and other factors have been mentioned but are of such importance that they should be repeated here. It is because of these various factors and the failure of investigators to take them into account that the literature contains many conflicting observations regarding the filtration of certain bacteria and other substances. For example, the filtration of *Leptospira icterohæmorrhagiæ* has never been settled satisfactorily. Some investigators state that this organism is filterable, and others that it is not. It becomes highly important then for reports to state definitely under what conditions filteration experiments are performed. Only in this way can accurate information be presented.

FILTERABLE BACTERIA

According to Wherry the bacillus of guinea-pig pneumonia measures 0.5 by 0.7 micron. This microbe is filterable through a Berkefeld V candle. The virus of pleuropneumonia in cattle is a small pleomorphic organism which is visible without doubt and is filterable through Berkefeld and Chamberland filters. According to Bordet this organism appears as a fragile spirochæte but shorter than the spirochætes of syphilis. Borrell found in stained preparations fork-shaped branchings and asteroid bodies and for this reason designated the organism *Asterococcus mycoides.* More recently Bridré and Donatien(3) have cultivated the virus of agalactia contagiosa which they state resembles the virus of pleuropneumonia of cattle in its morphology. This virus passes the Chamberland No. L1 filter but does not pass the L2 filter. While these two viruses closely resemble each other in many respects there are certain differences which have been pointed out by Bridré and Donatien (see Chapter V). The exact nature of these viruses remains unknown.

In 1926 Burnet(4) experimented with filtrates of organs removed from rats and guinea pigs dead from plague. From three filtrates he succeeded in getting cultures of *B. pestis.* Furthermore, one of these filtrates when injected into guinea pigs produced the infection. He states that the filterable form of *B. pestis* appeared as a granule proceeding from lytic disintegration of the bacillus.

During the same year Mellon(5) reported a filterable phase in the life history of *B. fusiformis* and related organisms. He states that the aërobic modification of *B. fusiformis* is a diphtheroid, the granules of which pass through a Berkefeld N candle but resist artificial cultivation. He suggests that the granule-containing filtrate may represent a filterable virulent form of the organism.

In 1910 Fontes(6) noted the presence of virulent filterable elements in the pus from a tuberculous abscess. In 1922 Vaudremer(7) found similar elements in cultures of tubercle bacilli. Calmette and Valtis(8) have confirmed these observations.

These authors have found that filtrates from tuberculous sputum or pus contain invisible elements that are virulent, leading to a typical tuberculosis. Furthermore, these authors have prepared filtrates from lymph glands of rabbits twelve days after an intraperitoneal injection of such filtrates and by intravenous injection of the filtrate have been able to demonstrate tubercle bacilli in the rabbit's spleen. Fontes believed that the so-called "Much granules" represent the invisible elements, but this idea has not been confirmed by experimental work.

Other authors have also reported filterable forms of tubercle bacilli. Mellon and Jost(9) prepared Berkefeld filtrates of tuberculous material and inoculated twenty-one guinea pigs. In twelve of these animals they found tubercle bacilli in direct smears prepared from the lungs. In three animals they demonstrated acid-fast bacilli in the inguinal and tracheobronchial lymph glands. No definite evidence of tuberculosis was found by histologic methods except in the lungs of two animals. Fabry(10) has also found tubercle bacilli in the glands and spleens of animals injected with a filtrate prepared with a fragment of human tuberculous lung. Filtrates prepared from the organs of these animals and injected into a normal animal resulted in a generalized tuberculosis. De Potter(11) has experimented with filtrates of avian tubercle bacilli and assumes that the pathogenic property of the filtrates is attributable to special forms of avian tubercle bacilli, analagous to those in filtrates of human or bovine bacilli. Fessle(12) has been unable to confirm the various reports that tubercle bacilli exist in filterable forms.

Fontes(13) in a recent study on the life cycle of bacteria concludes that—

> 1°—The bacteria studied are nucleate cells.
>
> 2°—The nucleus of these cells is dispersed, affecting chromidium forms which with the evolution of the bacterium toward reproductive activity mobilizes and condenses into granulations.
>
> 3°—The localization at the poles indicates an amitotic process, the details of which cannot be followed up because of the unsatisfactory nature of the apparatus used for observation.
>
> 4°—The irregular distribution of the chromidial substance inside bacterial cells, in the same manner as its regular distribution in cells of the same nature, seems to indicate that cells of the same species are able to divide and mutiply by different processes.
>
> 5°—The growth of these cells and their reproduction are closely connected with the growth and reproduction of the chromidial corpuscles.
>
> 6°—The growth and reproduction of these cells takes place through the emission of granules inside the protoplasm, disposing themselves for the ulterior division of the cell, or else through the emission of granules out-

side the organism studied, which will give rise to a new reticulum about to build up a new-formed cell.

7°—The growth and reproduction of these cells may take place in the length direction or laterally, providing for transverse planes of division (cocci, coli and dysentery bacilli) or longitudinal planes of division like the branching of a tree (diphtheria and tubercle bacilli and sometimes B. coli).

Sweany (14) in a recent study of the filterability of the tubercle bacillus finds that the granules sprout into actively motile bacilli and are non-acid-fast. Other granules he has found resemble cocci while others go through a gonidiform stage, in which the granules are held together by a matrix, and may sprout again in a way similar to that in free granules. These granules produce tuberculosis in guinea pigs and produce pure cultures of tubercle bacilli. This author believes that a virus exists in tuberculous lesions that is capable of passing through the finest Berkefeld filters and producing varying grades of disease in guinea pigs, depending upon the particular virus. He states that some viruses produce emaciation and death without any signs of tuberculosis. Others produce typical tuberculosis from which typical tubercle bacilli may be recovered.

Other species of bacteria are also said to produce filterable forms. Hadley (15) describes these under the caption "Filterable forms of bacteria secondary to lytic action." He says:

> We have now observed the influence of the bacteriophage in producing modifications in normal, sensitive culture and in normal cells, as also in "pure" S and R strains. We may now turn to its influence in causing the generation of filterable forms of bacteria. In the sections dealing with active dissociation in normal cultures it has been noted that such cultures may enter a stage of development in which they are filterable through Berkefeld or Chamberland candles that are able to hold back all microscopic forms. In most of these instances no signs of dissociation have been noted at the time. It is also clear, however, that accompanying that form of dissociation stimulated by the bacteriophage there often occurs a quick development of filterable bodies.

In 1922 d'Herelle (16) described filterable forms of the Shiga bacillus in filtrates of lysed cultures. Hauduroy (17) has reported filterable stages of *B. coli*, *B. typhosus*, *B. dysenteriæ*, and *M. aureus* under the influence of the lytic principle. Tomaselli (18) has made similar observations for *B. coli*. Hadley states:

> Some of these filterable forms were reported to yield a faint, opalescent growth in broth; and less frequently a delicate growth on solid mediums. It seems also that they may propagate in the invisible state. In still other instances they may revert to the original, but still resistant, cell type; or to a modified cell type (coccoid). In such cases the opalescent

> growth gradually gives place to a definite turbidity. One point of interest in these cases is that, whatever the morphologic type of organism submitted to lytic action at the beginning—rods or cocci—all show the same disintegrative trend toward granule formation; and in this end-state all meet on a common morphologic footing (d'Herelle and Hauduroy). . . . I believe that we may eventually find in this "cosmopolitanism" or convergence of the rough types, whether morphologic or serologic, and whether occurring in connection with simple dissociation or with transmissible autolysis, a deeper significance than now attached; and one bearing upon a number of at present inscrutable problems recently introduced into bacteriological literature.

Fejgin(19) has reported a filterable stage of the typhoid bacillus which is capable of inducing infection in guinea pigs. This virus was also obtained through the action of a lytic principle. This author has also obtained a secondary culture from proteus X 19 through the influence of a lytic principle. She also has produced secondary cultures in vivo with the typhoid virus by injecting this virus into guinea pigs. From the brains of these animals she has obtained minute coccobacilli, which differed from the normal cultures both biochemically and serologically.

D'Herelle(20) believes that the filterable forms of lysed bacteria such as have been described above are responsible for the inhibition of the increase in virulence of certain races of the bacteriophage. He states that if the interval between passages is prolonged this effect is not seen. Hadley states in this connection—

> If, as these workers also assume as a result of their more recent experiments, the lytic principle is bound up with some of these filterable forms, as well as with intact organisms of the resistant type, producing a symbiosis, such filterable forms would also be regarded, in the Bordet sense, as lysogenic.

The filterability of the streptococci is as yet obscure. It is believed by some investigators that certain strains of streptococci are filterable. Others maintain that only defective filters permit the passage of streptococci. In the preparation of scarlet-fever antitoxin several investigators have contended that the antitoxic serum produced in horses is a dual serum; that it is both antitoxic and antibacterial because a few streptococci pass through the filter with the toxin. This point is not entirely clear as yet. In general the evidence supports the view that some strains of streptococci will pass through certain filters, and the theory has been advanced that these organisms may be considered filterable at some stage in their life cycle. The entire subject needs further careful investigation before any preëmptory statements can be made in the matter. It is interesting to note in this connection that Evans and Freeman(30) have reported *Streptococcus* strains (presumably filterable) from encephalitis confirming previous findings by Rosenow.

Among the filterable forms should of course be mentioned *B. pneumosintes* of Olitsky and Gates (see Chapter IX), which is both visible and filterable; the virus of "Hühnerpest" propagated by Marchoux(21) and by Landsteiner and Berliner,(22) but which was invisible to these authors; the virus recently described by Noguchi as cultivated from the tick *Dermacentor andersoni* (see Chapter VIII), which is also invisible; and the filterable saprophytic bacteria which Borrel cultivated from water.(23) Hort, Larkin, and Benians(31) have reported the filtration of the meningococcus; Novy and Knapp(32) the filterability of the relapsing-fever spirochæte, while Pryor(33) in 1925 described the filtration of a "spore-forming coccus" which he thought to be the cause of scarlet fever.

We may conclude that filterable forms of several different species of bacteria exist and that they tend to occur in the form of granules. The relation of the bacteriophage to the production of these filterable forms of bacteria is not as yet fully understood. Further work should be done along this line of investigation.

FILTERABLE YEASTS

In 1926 Pappenheimer was investigating the cause of a peculiar disease of fowls which was associated with paralysis. In our laboratory filtrates were prepared from the emulsion of the sciatic nerve from one of these fowls. Tests for carbon dioxide (CO_2) production were performed with these filtrates according to the method described by McKinley and Coulter,(24) and after twenty-four to forty-eight hours it was found that considerable carbon dioxide (CO_2) had been produced in the tubes containing the filtrate when kept at incubation temperature. Pappenheimer made cultures from these filtrates and found smaller yeastlike organisms in the bottom of the tubes. While he was unable to produce the chicken paralysis with these cultures, the observation was interesting since we felt that this was an instance of a filterable yeast. In 1927 Lewis(25) reported a filterable yeastlike microörganism which he cultivated in the course of experiments with the virus of hog cholera. This organism he states is pleomorphic in extreme degree. He found both coccoid and bacillary forms at the lower limits of visibility.

The culture could be completely regenerated after filtration through Berkefeld N filters although these filters retained *Bacillus abortus* Bang. The culture is not pathogenic for swine. Lewis has named the organism *Schizosaccharomyces filtrans.*

FILTERABLE PROTOZOA

In 1902 Borrel(26) described filterable microörganisms which he obtained from water and which he believed belonged to the Protozoa. These organisms were apparently water flagellates. Schaudinn(27) two years later presented the idea that certain Protozoa might have a stage in their life cycle in which the organisms exist in the form of spirochætes. Novy and MacNeal(28) in 1904 described cultures of trypanosomes which were so small that they passed through very thin filters and recently Reich(29) has reported that the liver and spleen of animals infected with *Trypanosoma brucei* frequently yield filtrates which are infectious. *Leptospira icteroides* is filterable under certain conditions, but this organism is certainly not a stage in the life cycle of any other parasite. The postulate that filterable forms may represent a stage in the life cycle of various parasites is frequently referred to. The fact that so many different bacterial forms are apparently filterable and the tendency of these forms toward "granule formations" is strong evidence in favor of this idea. The study by Lewis of his filterable yeast is a case in point and, as he states, "seems to fulfill quite perfectly the requirements of this very general postulate."

The possibilities of filterable forms of bacteria, yeasts, and spirochætes have only been touched upon by investigators. That such forms do exist seems to be beyond doubt. From the standpoint of the mechanism of infection and communication of disease these observations are very important, and the entire subject should receive special study in the hope of ascertaining the fundamental principles involved in the production of these forms and their relation to disease processes.

BIBLIOGRAPHY

1. Bronfenbrenner and Muckenfuss, Proc. Soc. Exp. Biol. and Med. 24 (1926) 372.
2. Kramer, Journ. Infect. Dis. 40 (1927) 343.
3. Bridrè and Donatien, C. R. de l'Acad. des Sci. 177 (1923) 841. Ann. l'Inst. Pasteur 39 (1925) 925.
4. Burnet, Arch. de l'Inst. Pasteur de Tunis 15 (1926) 292.
5. Mellon, Journ. Bact. 12 (1926) 279.

6. FONTES (1910), quoted from Calmette and Valtis, Ann. de med. **19**: 553.
7. VAUDREMER (1922), quoted from Calmette and Valtis, loc. cit.
8. CALMETTE and VALTIS, Ann. de méd. **19** (1926) 553.
9. MELLON and JOST, Soc. Exp. Biol. and Med. **24** (1927) 743.
10. FABRY, Bruxelles-Médical **7** (1927) 596.
11. DE POTTER, C. R. de la Soc. biol. **96** (1927) 138.
12. FESSLER, Central. f. Bakt. **98** (1926) 148.
13. FONTES, Mem. de Inst. Oswaldo Cruz **18** (1925) 197.
14. SWEANEY, Am. Rev. Tuberculosis **17** (1928) 53, 77.
15. HADLEY, Journ. Infect. Dis. **40** (1927) 1–312.
16. D'HERELLE, The Bacteriophage. (Translation.) Williams and Wilkins Co., Baltimore (1922).
17. HAUDUROY, C. R. de la Soc. de biol. **91** (1924) 1209, 1325.
18. TOMASELLI, Contributo allo studio del batteriofargo (1923).
19. FEJGIN, C. R. de la Soc. de biol. **90** (1923) 1381; **91** (1924) 1106; **93** (1925) 365; **92** (1925) 1528; **93** (1925) 1530.
20. D'HERELLE, The Bacteriophage and its Behavior. Williams and Wilkins, Baltimore (1926).
21. MARCHOUX, Compt. rend. Acad. **147** (1908) 357.
22. LANDSTEINER and BERLINER, Centr. Bakt., 1, Abt., Orig. **67** (1913) 165.
23. BORREL, Compt. rend. Soc. biol. **54** (1902) 59.
24. MCKINLEY and COULTER, Proc. Soc. Exp. Biol. and Med. **24** (1927) 685.
25. LEWIS, Journ. Exp. Med. **45** (1927) 277.
26. BORREL, Compt. rend. Soc. biol. **54** (1902) 61.
27. SCHUDINN, Arb. k. Gsndhtsamte **20** (1904) 387.
28. NOVY and MACNEAL, Sixth Rep. Michigan Acad. Sci. (1904) 180, cited by Novy and Knapp, Journ. Infec. Dis. **3** (1906) 291.
29. REICH, Journ. Parasitol. **10** (1923–24) 171.
30. EVANS and FREEMAN, U. S. P. H. S. Pub. Health Rep. **41** (1926) 1095.
31. HORT, LARKIN, and BENIANS, Brit. Med. Journ. **1** (1915) 541.
32. NOVY and KNAPP, Journ. Infect. Dis. **3** (1906) 291.
33. PRYOR, Am. Journ. Pub. Health (1925) 847.

CHAPTER XVII

INTRACELLULAR INCLUSIONS IN FILTERABLE VIRUS DISEASES

The exact nature of the intracellular inclusion bodies is not known. That they are uniformly associated with many of the diseases of filterable virus origin is now a well-established fact. Indeed, in many diseases they are regarded as pathognomonic. In some diseases both the filterability of the virus and the presence of inclusion bodies have been demonstrated. In others inclusion bodies have been found but the nature of the virus remains obscure. Based upon the presence of inclusion bodies these diseases are thought to be caused by filterable ultramicroscopic viruses even though the causative agents cannot be determined. In other diseases filterable viruses have been demonstrated but no inclusion bodies are found.

Most of the inclusion bodies have been named for their discoverers; for example, Negri bodies in rabies and Guarnieri bodies in variola and vaccinia. As has been pointed out in the chapter on mosaic diseases of plants the inclusion bodies when first discovered were thought to represent parasites. This has been true for practically all of the filterable virus diseases in which inclusion bodies have been found. The theory generally in acceptance twenty-five years ago was that these bodies represented protozoans having complicated life cycles. In 1907 Prowazek(1) suggested a modification of this idea. According to Prowazek's theory the inclusion bodies represent the causative agent embedded in reaction products of the cell. This author designated these bodies Chlamydozoa (armored or cloaked animals). In 1912 Lipschütz(2) suggested the term Strongyloplasmata for this group of bodies. Later, in 1921, Lipschütz suggested a classification as follows:

1. Cyto-oikon group in which the inclosures lie in the cytoplasm. Examples of this group are trachoma, molluscum contagiosum, fowl-pox, and sheep pox.
2. Karyo-oikon group in which the inclosures lie in the nucleus. Examples of this group are warts and herpes.

3. Cyto-karyo-oikon group in which the inclusion bodies lie in both the cytoplasm and nucleoplasm. Examples of this group are variola and para-vaccinia.

In a recent review of the cell inclusions Findlay and Ludford(3) state, "It is doubtful whether in the present state of our knowledge the term 'Chlamydozoa' can be justified as a cloak for anything except our ignorance." According to Lipschütz the inclusion bodies consist of the virus itself and are found within the affected cell as a result of proliferation of the infectious agent. In connection with this idea Goodpasture(4) states:

> While this hypothesis is a most useful one and has much to support it, further evidence is necessary to establish its truth. One cannot rely entirely upon the morphology of minute components of such structures to establish with certainty their parasitic nature. But whether or not it will eventually be proved that the inclusions and the virus are identical, nevertheless much can be learned by clearly recognizing a constant association of a characteristic type of inclusion with any particular infection, and by identifying it with the lesion so that a diagnosis of the infection on this basis may be possible.

This author, for example, has shown that by injecting the herpes virus into the right masseter muscle in rabbits, the virus passes up the axis cylinder of the nerve trunk and demonstrable herpetic lesions are to be found within the motor nucleus of the right fifth cranial nerve. Goodpasture believes it quite probable that the virus grows within the axis cylinder and so propagates itself to its central termination. That the inclusion bodies described in such an experiment are directly associated with infection with the herpes virus is beyond question for they are produced at will and it is possible to prophecy their future location depending upon the portal of entry.

We have seen in the previous chapter that Smith(5) was able to confirm the observations of Goldstein in regard to the vacuolated bodies found in the epidermal and hair cells of leaves of mosaic tobacco plants. Smith was unable to discern any autonomous movements in the vacuolated bodies and in only one instance observed a limiting membrane. Here again the inclusion bodies are regarded as the product of a reaction between the virus and the cytoplasm of the cell. They do not occur in normal healthy tobacco plants but are uniformly found in these plants when infected with mosaic. It is evident then that inclusion bodies are associated not only with filterable virus diseases of animals but are found also in filterable virus diseases of plants.

It is proposed in this section to describe several of the types of inclusion bodies which have been associated with filterable virus diseases. Among the diseases for which inclusion bodies have been described are the following:

1. Diseases of man.
    - Variola.
    - Vaccinia.
    - Paravaccinia.
    - Alastrim.
    - Molluscum contagiosum.
    - Trachoma.
    - Varicella.
    - Verruca.
    - Inclusion blennorrhœa.
    - Herpes.
        - Simplex (febrilis).
        - Genitalis.
        - Zoster.
    - Scarlet fever.
    - Chronic epidemic encephalitis (in a child).
    - Acute encephalitis.
    - Inclusions in diseases of unknown origin.
2. Diseases of animals.
    - Sheep-pox.
    - Foot-and-mouth disease.
    - Rabies.
    - Distemper.
    - Borna disease.
    - Swine fever.
    - Salivary-gland disease of guinea pigs.
    - Virus-III infection of rabbits.
    - Kurloff bodies in guinea pigs.
    - African horse sickness.
3. Diseases of fowls.
    - Fowl pox.
    - Fowl plague.
    - Macfie's disease of fowls.
4. Diseases of fishes.
    - Lymphocystic disease of fishes.
    - Carp pox.
    - Epithelioma of *Barbus*.
5. Diseases of insects.
    - Polyhedral diseases of insects.
        - Wilt disease of gypsy-moth caterpillar.
        - Wilt disease of the European nun-moth caterpillar.
        - Jaundice of silkworms.
    - Nuclear disease of the caterpillar of the cabbage white butterfly (*Pieris brassicæ*).
6. Diseases of plants.
    - Mosaic diseases of tobacco, sugar cane, corn, potato, petunia, bean, tomato, and mosaic-free plants.

INTRACELLULAR INCLUSIONS IN VARIOLA, VACCINIA, ALASTRIM AND PARAVACCINIA

Renaut(6) in 1881 first described inclusion bodies within the epithelial cells of variolous lesions. Guarnieri(7) later described these bodies, and since his report in 1892 these intracellular bodies have borne his name. Guarnieri regarded these bodies as protozoans and suggested the name *Cytoryctes vacciniæ* for them. It was also believed at this time that the inclusion bodies represented a stage in a complicated life-cycle of Protozoa. Evidence to prove this concept was brought forward by Councilman, Magrath, and Brinckerhoff,(8) Calkins,(9) and Prowazek.(2) Copeman and Mann,(10) however, thought that these bodies arose from some preformed cell constituent, while Ewing(11) believed that the vaccine bodies originated from nuclear material of the cell. The vaccine bodies are found in the epithelial cells of affected parts of the skin and also between the cells. They measure only from 0.2 to 0.5 micron in size, are Gram negative, and stain with Loffler's flagella stain and with Giemsa's. Borrel(12) described similar bodies in sheep pox in 1903. Inclusion bodies have been described for alastrim by Castellani and Chambers, while in paravaccinia both cytoplasmic changes and intranuclear inclusions have been described by Lipschütz which are similar to those found in herpes infections. There have been many conflicting views regarding the nature and origin of these bodies. In 1920 Böing(13) reported that the vaccine virus itself could be demonstrated in the cytoplasm of the cell and at times within the nucleus as minute granules which stain a deep red with Azur I. On the other hand Woodcock(14) has brought forward the old concept that the inclusion bodies are the result of the digestion of red blood corpuscles. Others have suggested that the vaccine bodies are the result of leucocytic migration or leucocytic fragmentation. In 1922 Cowdry(15) attempted to show that the vaccine bodies are formed from the normal constituents of the cell and that these substances increase in quantity under the stimulus of vaccination. Supravital staining has demonstrated blue-staining droplets within larger pink-staining masses. There is no evidence that these bodies contain microörganisms. Prowazek has designated the small bodies found in pox lesions in man and in vaccinia as Chlamydozoa (armored or cloaked animals), but they are also referred to as Prowazek's elementary bodies. Later these bodies become larger and are thought to represent a later stage in the development of the virus. The larger forms are referred to as Prowazek's initial bodies. Lipschütz(2) has designated them as *Strongyloplasma variola-vaccinæ.*

The Guarnieri bodies are spherical or half-moon-shaped bodies thought to consist of chromatin or plastin substance. They are usually found lying close to the nucleus. In fresh preparations some investigators have described amœboid movement, but Schütz(15) in a recent paper states that they are decidedly not amœbalike. This author believes that they are true cytoplasmic structures which arise as a result of infection of the cell by the vaccinia virus. According to this view it is reasoned that the minute infectious agent, the vaccinia virus, enters the cell substance and there becomes inclosed by a plastin substance which is produced by the plasma of the cell as a protective reaction against the invader. Further, the vaccine body again breaks down, the initial body passes into the plasma of the cell, there disseminates and separates into elementary bodies. The elementary bodies may then reach the outside world again after the epithelial cell is destroyed and are capable of again infecting a susceptible host.

The view which seems to be most acceptable at present is that the vaccine bodies arise from the normal constituents of the cell as a direct result of the infection. The virus may or may not be present in the inclusion body. This is not known and at present there is apparently no method known by which this can be determined. It is known from culture experiments with the vaccinia virus that living cells are essential in the medium in order to obtain multiplication of the virus. The virus does not multiply upon dead organic matter. This suggests to the writer still another possibility regarding the origin and nature of these bodies. The virus after entering the cell finds there certain substances which are essential for its existence and multiplication. Through the functional activity of the virus these substances or these elements are withdrawn from the normal cell constituents to the virus. The presence of this substance in close proximity to the virus in addition to serving as nutritive material may also assist in creating physical conditions favorable for multiplication of the virus. As the virus multiplies more of this substance is needed and as a result of virus multiplication and accumulation of the specific substance or substances the vaccine body becomes larger. That the vaccine bodies do become larger is well known. If such were the case it should be possible to provoke the formation of vaccine bodies in tissue culture provided, of course, that the specific substance or substances so necessary are present in the cell in sufficient quantity and other sources of supply such as exist in vivo and may not exist in vitro are depended upon for constant replenishing. In case only a limited supply of the material essential for multiplication of the virus is present in the cell, one would expect the virus to multiply to a certain point, then stop. This is apparently just what happens in tissue culture, and repeated transfers to fresh tissue-culture medium are necessary in order to continue propagation of the virus. Parker and Nye (17) cultivated the vaccinia virus for one hundred thirty-two days in tissue culture, but the virus was not demonstrable after one hundred ninety-eight days. Gracium and Oppenheimer (18) cultivated the vaccinia "granules" in vitro with embryonic tissues for seventy-one days as tested by rabbit corneal inoculation. As Parker and Nye have pointed out in connection with their culture work with the herpes virus the tissue cells themselves may be damaged by the infectious agent and as a consequence their growth is checked, which results in the eventual death of the virus.

As the cultivation of the filtrable viruses, such as vaccinia virus, develops we believe that more light will be thrown upon the origin and nature of the inclusion bodies. It seems highly probable that the infectious agents are present in the inclusion bodies and that the formation of the inclusion bodies is closely related to growth and multiplication of the virus.

INCLUSION BODIES IN OTHER POX DISEASES

The presence of inclusion bodies in sheep pox has already been mentioned. Borrel has shown that bodies like Guarnieri bodies are present in the infected epidermal cells in this disease. Such bodies have also been described by Bosc,(19) Paschen,(20) and others. The virus of sheep pox has not been cultivated. Pox disease also occurs in swine, goats, horses, and fowls. Gins and Rickert(21) consider all forms of pox as originating from human pox since they claim to have changed human, swine, goat, and sheep pox into cowpox by passage through rabbits. Inclusion bodies have not been reported for pox disease in goats or swine. Intracellular inclusions have been described for horsepox and fowl pox. The relationship between fowl pox and avian diphtheria has not been definitely determined. Some investigators believe that these diseases are identical but are characterized by different manifestations. (See Chapter XI.) In 1873 Bollinger(22) and in 1881 Rivolta(23) described the flagellate *Cercomonas gallinæ* in avian diphtheria and the cytoplasmic inclusions which are present in the epidermal cells of fowl pox. These bodies were thought to be Protozoa. In fowl pox gregarines were thought to be the etiological agents. Later, various bacterial forms were isolated from cases of the disease, but the true virus was definitely shown to be filterable by Marx and Sticker(24) in 1903. In the same year Michaelis(25) described the cytoplasmic inclusions in some detail. The inclusion bodies or "chickenpox bodies" which are found in the epithelial cells have been designated Chlamydozoa by Prowazek and Strongyloplasma by Lipschütz. Emulsions as well as the filtrates from epitheliomas contain small, spherical, nonmotile bodies, (0.25 micron in size) which stain by Giemsa's with Loeffler's flagella stain, and with Ziel's fuchsin. Prowazek believed that these small bodies may penetrate the epithelial cells and there produce reaction products which result in so-called cellular inclosures, or pox bodies. The theory has also been advanced that the pox bodies are thrown off by the nucleus of the cell and that the disease is due to a toxin thrown off by the epithelial cell. Bordet has cultivated small granules which measure about 0.2 micron, from diphtheritic material, on blood-gycerin-potato agar, but these cultures while they produce diphtheritic membranes do not produce epitheliomata on the skin. The cytoplasmic inclusions in fowl pox are said to be partly lipoidal in character, but they have been little studied and very little is known concerning their true nature or their relation to the disease process.

Inclusion bodies have also been described in a form of pox affecting the carp by Loewenthal,(26) but they have been little studied and very little is known concerning them. (See Chapter XIII.)

INCLUSION BODIES IN HERPES INFECTION AND IN ENCEPHALITIS

Lipschütz has reported intranuclear inclusions in all forms of herpes in man, while Luger and Lauda,(27) Goodpasture, and others have described similar bodies in experimental herpes infection in rabbits. Other investigators, Da Fano(28) and Cowdry and Nicholson,(15) have described nuclear and cytoplasmic granules in experimental encephalitis in rabbits which are not specific for herpes and differ from those described by Lipschütz. Levaditi(29) has also described inclusions in experimental encephalitis in rabbits which he designates "neurocorps encephalitiques" but there is grave doubt that the virus Levaditi experimented with is any other than a herpes virus.

The inclusions described by Lipschütz are without doubt specific since they are also found in the epithelial cells of the inoculated rabbit's cornea. These bodies are found only in the actual seat of the lesion, and in herpes simplex and herpes genitalis they have been found to appear in serial inoculation. The herpetic inclusions are found almost exclusively with the nucleus and not in the cytoplasm of the cell. In describing the herpetic inclusions in rabbits Goodpasture states:

> It is in ganglion and neuroglia cells of the central nervous system that it has been possible to study the inclusions to best advantage. In motor ganglion cells they are readily distinguishable from the coagulated nucleoplasm or normal cells by their size and configuration. The material of which they are constituted greatly increases in amount and may form

a compact crescent or ring about the nucleolus, and eventually it completely fills the intranuclear space which coincidently enlarges. In ganglion cells there is not the same tendency for fluid to accumulate within the nucleus as in other types of cells in herpetic lesions, and in consequence the intranuclear body may not be separated from the nuclear membrane by a clear zone. The nucleolus shows evidences of disintegration when the inclusions are well developed. It loses its symmetrical contour, becomes vacuolated or breaks up into irregular granules. The chromatin is collected about the nuclear membrane. The cytoplasm of such a ganglion cell shows chromatolysis partial or complete. In other cells of the rabbits in tissues acutely infected with the virus of herpes simplex, an intranuclear material with staining properties identical with that of herpetic inclusions in ganglion cells presents an even more conspicuous structure.

Goodpasture was able to produce herpetic inclusions within twenty-four hours following injection of herpes virus directly into a corpus luteum of early pregnancy in rabbits. The ovary was also found to be very rich in virus. This author states that the time element is a most important factor in producing herpetic inclusions since the inclusions disappear rapidly in lesions caused by the virus and in direct proportion active virus diminishes.

Lipschütz has reported the following staining properties of the herpetic inclusions:

Stain.	Inclusions.	Nucleoli.
Giemsa.	Red.	Dark blue.
Hæmatoxylin-eosin.	Dark red.	Blue black.
Heidenhain's iron-hæmatoxylin.	Yellow gray.	Black.
Pappenheim.	Green or blue.	Red.

Of the origin and nature of herpetic inclusions we know little. Interest has been sustained in the study of the herpes viruses particularly because of the opinion in some quarters that the herpes virus is the cause of epidemic encephalitis. Da Fano(29) has described inclusion bodies in both acute encephalitis and in chronic epidemic encephalitis. These bodies he designates "minute bodies" which he found only rarely in the nerve cells but commonly in the cytoplasm of polymorphocytes and in a few instances they were noted in lymphocytes. These bodies, however, are not to be confused with herpetic inclusions.

In Borna disease or meningo-encephalo-myelitis of horses (see Chapter IV), Joest and Degen(30) in 1909 described intranuclear inclusions in the ganglion cells of the brain. In the ganglion cells of the cornu ammonis the oxyphilic corpuscles described by these authors are noted in large numbers. These formations were found in cells which presented a marked rarification of the karyoplasm but were not found in degenerated cells. These bodies were also in evidence in the pyramidal cells of the cerebrum, medulla, anterior horns of the spinal cord, and in the nerve cells of the spinal ganglia. In a recent paper Nicolau and Galloway(31) describe similar bodies in the large ganglion cells of the cornu ammonis of horses having this disease.

INTRACELLULAR INCLUSIONS IN VARICELLA AND VIRUS-III INFECTION OF RABBITS

In 1906 Tyzzer(32) described inclusion bodies in the cells of varicella lesions in man. Both nuclear and cytoplasmic inclusions were found by this author. The nuclear inclusions stain red with eosin-methyline blue and range from 1 to 6 microns in diameter. The cytoplasmic inclusions stain deep purple and in some instances central granules are present. The lesions produced upon the rabbit's cornea with varicella material do not contain inclusions as in the case of vaccinia virus. Bertarelli(33) claims to have produced skin lesions in rabbits with varicella material and to have demonstrated intracellular inclusions in the epidermal cells. Kesselitz and Mayer(34) have described inclusions in the cytoplasm of epithelial cells of varicella lesions, while Gins(35) also claims to have produced skin lesions in rabbits. Rivers(36) has described intranuclear inclusions in the cells of the testis of monkeys in which he had previously inoculated varicella material. These inclusions were observed in the glandular cells of the testicle removed on the sixth day and stained with eosin. In a second monkey showing a similar gross reaction to the inoculation the testicles were removed on the eighth day but no inclusions were found.

Virus III was discovered by Rivers and Tillett(37) while attempting to produce chicken pox in rabbits. The agent produces gross as well as microscopic lesions in the cornea, skin, and testicles of rabbits. Microscopically the lesions on the skin and cornea of the inoculated rabbits showed the presence of intranuclear inclusion bodies. Later Andrews and Miller(38) found this virus in the testicles of apparently healthy rabbits.

INCLUSION BODIES IN MOLLUSCUM CONTAGIOSUM

Wile and Kingery(39) not only demonstrated that the virus of molluscum contagiosum is filterable but also succeeded in producing experimentally in human beings typical tumors with the sterile filtrate of typical lesions. These authors believe that the molluscum body develops late in the stage of evolution of the tumor and further that it represents a degenerative stage in this evolution. MacCallum(40) in 1892 suggested that the bodies were formed as a result of nucleolar extrusion. This phenomenon, however, as pointed out by Findlay and Ludford,(3) is usually associated with normal keratinization in most epidermal cells. The exact nature of these bodies is unknown. In the past they have been mistaken for parasites but this concept receives little serious thought to-day. (See Chapter III.)

INTRACELLULAR INCLUSIONS IN RABIES

In 1903 Negri(41) first described cytoplasmic inclusions in the ganglion cells of the brain in rabies. These bodies, the so-called Negri bodies, now considered diagnostic of the disease, were first thought to be protozoan parasites. There are few who adhere to this belief to-day. Minute basophilic structures are found within the Negri bodies which some investigators believe represent the true virus of rabies. The filterability of the infectious agent in rabies is beyond question. The virus then must be exceedingly small. The virus of rabies is supposed to have been cultivated by Noguchi(42) in 1913, but this work has not been confirmed and the descriptions given for the minute pleomorphic bodies cultivated by this author remind one somewhat of yeast cells. The Negri bodies are small, round, oval or three-cornered inclusions measuring about 1 to 27 microns in length and 1.5 to 5 microns in width. Within the bodies are found very small, refractile, and sharply outlined granules. Volpino(43) has described these bodies as consisting of a hyaline ground substance in which sometimes very small, marginal, and at other times, larger, central formations seem to be embedded, which contain very fine ring-, rod-, or dumb-bell-shaped inclosures. Manouélian and Viala(44) believe that the true parasite is a rod-shaped body which agglutinates and degenerates to form Negri bodies. These authors believe the "Encephalitozoon rabiei" is closely related to the so-called "Encephalitozoon cuniculi" found in spontaneous encephalitis of rabbits. Goodpasture has proposed the name "lyssa bodies" for these inclusions and suggests that they are formed by the neurofibrillar material of the cell. Stained by the Giemsa method the Negri bodies are light blue in contrast to the darker and more violet cell bodies. By the Mann method the nerve cells are stained pale blue, and in their cytoplasm the small oval bodies are found stained deep pink. The Negri bodies are considered to be specific for rabies, though Acton and Harvey(45) believe that they are associated with nucleolar extrusion. Their exact origin and nature are still unknown.

INTRACELLULAR INCLUSIONS IN SCARLET FEVER

In 1904 Mallory (46) reported certain protozoan-like bodies in the epidermal cells of four cases of scarlet fever. Later this author withdrew his protozoan theory of the etiology of scarlet fever and described a bacillus (see Chapter V) as the cause of the disease. Peculiar bodies have also been found by Döhle (47) in the polymorphonuclear leucocytes in scarlet fever. These bodies closely resemble cocci. Macewen (48) has also described these, while Hoefer (49) has reported certain inclusion bodies found in cases of the disease. More recently Smirnowa-Zamkowa (50) has described certain oxyphil bodies in the tissues and bile of persons dying of scarlet fever. These bodies stain with eosin, and cultures which were positive through several transfers when injected into rabbits gave rise to similar bodies in several animal passages. These bodies are apparently attached to the red blood cell and are highly refractile. This author believes the bodies he has described to be the actual virus of scarlet fever and that they prepare the soil for invasion of the streptococcus universally present in the disease. Such interpretations must be viewed with conservatism in view of the convincing evidence which during the past few years has been presented for the streptococcus as the cause of this disease. As pointed out by Findlay and Ludford similar bodies are found in most diseases caused by pyogenic organisms and they are probably the products of cytoplasmic degeneration.

INTRACELLULAR INCLUSIONS IN TRACHOMA AND INCLUSION BLENNORRHŒA

Cytoplasmic inclusions were first described in trachoma by Halberstädter and Prowazek (51) in 1907. These bodies appear as reddish violet, round or ovoid granules when stained by the method of Giemsa. According to Noguchi and Cohen (52) a clear halo surrounding the bodies is sometimes noted. As the number of bodies increase they become surrounded by a blue-staining substance, plastin. Scrapings from the conjunctiva injected into the eye of the orang-utan produced conjunctivitis associated with the appearance of similar inclusion bodies. Later Halberstädter and Prowazek found similar inclusions in cases of uncomplicated blennorrhœa neonatorum and their specificity was questioned. Solovief (53) has also described small round blue-staining bodies in the epithelial cells of the conjunctiva which are not specific for trachoma. Herzog(54) has suggested the theory that the gonococcus is transformed into small forms within the epithelial cells and that the so-called trachoma bodies are in reality changed gonococci. Williams(55) has regarded the inclusion bodies in trachoma as degenerated forms of the Koch-Weeks bacillus.

In 1913 Noguchi(56) claimed to have cultivated the trachoma bodies, although he was unable to induce trachoma in monkeys with his cultures. Recently this author has cultivated a Gram-negative bacillus from trachoma cases and has produced a trachomalike condition in monkeys with this culture.

A disturbing factor in connection with the so-called trachoma bodies is the discovery of similar bodies in nongonorrhœal conjunctivitis of the newborn. Linder(57) inoculated two baboons with pure inclusion blennorrhœa material and obtained a clinical and histological picture which he states cannot be distinguished from trachoma. Wolfrum(58) has inoculated similar material into human beings with the same result. Findlay and Ludford present three possibilities in regard to nongonorrhœal conjunctivitis of the newborn; namely, it is a disease due to a virus allied to that of trachoma, the virus is the same in both diseases, or trachoma bodies are due to the virus of nongonorrhoal conjunctivitis of the newborn. A fourth possibility occurs to the writer; namely, that the inclusions may be the result of some secondary infection.

According to Solovief the trachoma bodies are the result of nuclear degeneration. It would perhaps be more conservative to state that their origin and nature are unknown.

INTRACELLULAR INCLUSIONS IN VERRUCA

Intranuclear inclusions were described in warts by Lipschütz(59) in 1924. Similar inclusions have been found in infective warts of dogs by Ullmann.(60) That warts are caused by a filterable virus has been demonstrated by Wile and Kingery.(61) The inclusions in warts have been little studied and little is known concerning them. They are basophilic and according to some authors are said to represent a stage in the degeneration of the nucleoli.

INTRACELLULAR INCLUSIONS IN DISEASES OF UNKNOWN ORIGIN

Intracellular inclusions have been described in a variety of diseases of obscure origin. Jesionek and Kiolemenoglou(62) in 1904 reported protozoan-like structures in the organs of an eighth-month syphilitic stillborn fœtus. Groups of from ten to forty of these bodies were found in the connective tissue of the cortex of the kidney. They were also present in the liver and lungs. Ribbert(63) has reported a similar case in which similar bodies were present in the kidneys and parotid gland. Loewenstein(64) found inclusion bodies in the parotid glands of four children. These inclusions were thought by Ludwig to represent protozoans, either coccidia or other Sporozoa. Pissano (65) also found inclusion bodies in the nuclei of the cells of the kidney, liver, and lungs of a stillborn syphilitic fœtus, while Mouchet(66) described similar bodies in the bile-ducts of an eighth-day syphilitic infant having icterus. Smith and Weidman(67) described intranuclear bodies, which they regarded as amœbæ, in the liver and kidney of a newborn infant. Later these authors reported a similar case of intranuclear bodies in a child 2 months old, dying of pneumonia and having a negative Wassermann.

In 1921 Goodpasture and Talbot(4) reported the presence of acidophilic intranuclear bodies in the lungs, bronchi, and glomeruli of the kidneys of a child 6 weeks old. This child had had green stools from birth, glucose in the urine, œdema of the feet, and a cough. These authors suggest that the inclusion-containing cells originate from the mononuclear cells situated just outside the endothelium of the small veins and capillaries. De Lange(68) has reported a similar case, while Müller(69) has described three cases in which inclusions were found.

Von Glahn and Pappenheimer(70) in 1925 described intranuclear inclusions in the cells of the intestine, liver, and lung of a man, 36 years old, dying with abscess of the liver, ulcerative colitis, pleurisy, and lobar pneumonia. It was not possible for these authors to demonstrate granules within these inclusions. In general their staining reactions were similar to those obtained with herpetic inclusions. Von Glahn and Pappenheimer state in regard to these inclusions:

> Since there is no possibility of carrying on an experimental study with the material from this case, an interpretation of the nature and significance of the inclusions must for the present rest upon the basis of similar observations recorded by others. There can be no doubt that the inclusions are identical in their morphology and staining reactions with the bodies seen by previous observers in the viscera of infants, and by Lipschütz and others in the tissues of spontaneous and experimental herpes, and in the various neural and visceral lesions produced by the herpetic and related viruses.

These authors suggest that the bodies described by them, including those occurring in herpes and related conditions, represent merely a peculiar form of nuclear degeneration, not produced by a specific virus or the bodies indicate the localization of the herpes virus or a similar virus within the nucleus of certain visceral cells.

INTERCELLULAR INCLUSIONS IN FOOT-AND-MOUTH DISEASE AND IN SWINE FEVER

Gins(35) has described intranuclear bodies in foot-and-mouth disease in the lesions on the tongue of the guinea pig. These inclusions are about 1.5 microns in size and stain deep red with Giemsa's. In 1908 Terni(71) observed a protozoan-like body (*Cytorhyctes*), which measured about 0.5 micron, in the vesicular lymph and in the internal organs of over four hundred cattle affected with foot-and-mouth disease. More recently (1926) Ruhle(72) reports inclusion bodies similar to those described by Gins in the epithelium of the tongue of the healthy guinea pig and cow. According to Gins the inclusion bodies described by him are found early in the course of the disease, usually two or three days after infection. The significance of these inclusions remains undertermined, but their occurence in normal epithelium would indicate that they are not specific.

All attempts to demonstrate the virus of hog cholera or swine fever under the highest magnification or to cultivate it in artificial media have failed. Uhlenhuth and Böing(73) have described certain cellular inclusions in smears prepared from the conjunctiva. These inclusions represent very fine granules similar to the so-called trachoma bodies in man to which reference has already been made. Such inclusions have been considered by Halberstädter and Prowazek as parasites. Their exact nature is unknown.

CELLULAR INCLUSIONS IN FOWL PLAGUE AND MACFIE'S DISEASE OF FOWLS

Rosenthal,(74) Kleine,(75) and Schiffmann(76) have described small ring-shaped bodies inside and outside the brain cells in fowl plague. These inclusions are said to be not unlike the Negri bodies. They may also be round or oval, and their true nature has not been determined. Prowazek(77) did not confirm these findings but described in the brain tissue small dumb-bell-shaped forms which measure about 1 to 1.5 microns. These forms frequently lie close to the red blood cells and are also demonstrable in filtrates of material known to contain the virus. Prowazek believed that these bodies were Chlamydozoa.

Macfie(78) and Adler(79) have described a disease of fowls in Nigeria and Palestine the symptoms of which are similar to those of fowl spirochætosis. Within the leucocytes they found granular bodies which resemble the nuclear material of malaria parasites when stained with Giemsa's. These granules were not present in mast cells or in eosinophiles. The authors regard these inclusions as Chlamydozoa. No bacteria or protozoans were found to explain the cause of the disease although the blood was infective for healthy fowls.

INTRACELLULAR INCLUSIONS IN AFRICAN HORSE SICKNESS

African horse sickness is believed to be an insect-borne filterable virus disease. Kuhn(80) has described the presence of cellular inclusions in the renal epithelium, but little is known concerning them.

INTRACELLULAR INCLUSIONS IN THE SALIVARY GLAND AND BLOOD CELLS OF GUINEA PIGS

In 1920 Jackson(81) first described the presence in the salivary glands of guinea pigs of an intracellular body which she regarded as a protozoan parasite. According to Jackson the protozoan most often appears in the cells of the ducts of the salivary gland as an encysted, round or oval structure. Small round bodies were described by this author in the peripheral zone of the parasite which she thought represented merozoites. Goodpasture and Talbot(4) found similar bodies in the salivary glands of guinea pigs which they regard as identical with inclusion bodies previously described in infants. More recently Cole and Kuttner(82) studied the salivary glands of seventy-five guinea pigs and found that 84 per cent contained these bodies. These authors identified these cells as swollen epithelial cells, the nuclei of which contain a mass of granular material that is definitely acidophilic. By transmission experiments they were able to conclude that they were dealing with a virus disease. The cytoplasm of the cell stains light blue, the nuclear membrane stains deeply with basic dye, and near the center of the nucleus is a mass stained red. This mass may occupy one-fourth of the nuclear space or fill it entirely except for a narrow halo between it and the membrane. This clear area may contain irregular masses which stain deeply with the basic dye. (See Chapter VIII.)

In certain of the large mononuclear cells in the blood of guinea pigs acidophilic inclusions have been found in the cytoplasm. These are known as "Kurloff bodies" and are regarded by some authors as Chlamydozoa. Similar inclusions have also been noted in sheep, rats, and certain fowls.

INTRACELLULAR INCLUSIONS IN CERTAIN DISEASES OF FISHES

LYMPHOCYSTIC DISEASE OF FISHES AND EPITHELIOMA OF BARBUS

In the lymphocystic cell the nucleolus is quite distinct, and in old cells it is acidophilic while the cell membrane is basophilic according to Weissenberg. (83) Within the plasma are seen vacuoles, particularly around the nucleus. Between the vacuoles and around their outer surface is seen a pronounced granulation. Besides granules one also notes very fine strands. Both granules and strands are apparently lipoid in nature. Weissenberg first thought that the granules represented the virus causing the disease but later withdrew this opinion. In these cells is found a network body which Weissenberg believes is a specific reaction product comparable to the cellular inclusions of the Chlamydozoa diseases. (See Chapter XIII.)

Keysselitz (84) has studied the histology of the epithelioma, as it occurs in *Barbus,* in sections stained with Ehrlich's hæmatoxylin and Breinl's stain for trypanosomes, and has demonstrated minute bodies which he believes are Chlamydozoa. He states that these nuclear bodies are comparable to the cellular inclusions of vaccinia, variola, epithelioma of fowls, molluscum contagiosum, trachoma, and rabies.

INTRACELLULAR INCLUSIONS IN DISEASES OF INSECTS

In sections through sacbrood-diseased larvæ of bees White (85) has found that the bulk of the body is composed of fat tissue. The fat cells are irregular in outline and possess an irregular-shaped nucleus. Black-staining spherical bodies are to be found within the cells. This gives the larvæ a granular appearance. The nature of the dark-staining bodies in these cells is not definitely known. Possibly they may be related to inclusion bodies found in several of the filterable virus diseases.

Among the so-called polyhedral diseases of insects are the wilt diseases of the gypsy-moth caterpillar and the European nun-moth caterpillar. Some investigators believe that these two diseases are identical. Polyhedral bodies are found in both. The average size of these bodies is from 1 to 6 microns. They are shaped like a polyhedron with rounded angles and are never spherical. They are highly refractive and possess a center denser than the periphery according to Glaser.(86) In fresh preparations very minute dancing granules are to be seen which may come from the polyhedral bodies. Glaser suggests that these dancing granules may be particles of degenerated chromatic or achromatic substance, but he is inclined to the view that they represent extremely minute microörganisms and that they may represent the vegetative stage of the polyhedral bodies or the polyhedral bodies may be a secretion of a minute organism contained within. He says, "as long as there is no evidence, however, that the polyhedral bodies are directly related to the filterable virus or to the little granules, the view that they are reaction products appeals more strongly."

Polyhedral bodies are also found in jaundice of the silkworm. These were first thought to be parasites by early investigators. A complete description of these bodies will be found in Chapter XII. Their exact nature is unknown.

According to Paillot(87) there is a nuclear disease of the caterpillar of *Pieris brassicæ* (large white cabbage butterfly) associated with the presence of refringent rings in the cytoplasm of the blood and fat cells. It is thought that the refringent rings are derived from the mitochondria.

INTRACELLULAR INCLUSIONS IN MOSAIC DISEASES OF PLANTS

Various intracellular bodies have been described in mosaic diseases of plants and in normal plants. Matz(88) has described such bodies in mosaic-diseased sugar cane. Kunkel(89) found them in corn; Palm,(90) Goldstein,(91) and Rawlins and Johnson(92) in tobacco infected with mosaic; McKinley, Webb, and Eckerson(93) in wheat rosette, and Smith(94) in potato mosaic. Nelson(95) on the other hand has described biflagellates and trypanosomes in the tissues of plants, while Duggar and Karrer(96) found similar structures in both healthy and diseased plants. The significance of cellular inclusions in plants is not known. It seems likely, however, that some of the inclusions that have been described may bear the same relation, whatever this is, to the mosaic-disease process that other intracellular bodies are thought to bear to filterable virus diseases as they occur in other forms of life. (See Chapter XIV.)

BIBLIOGRAPHY

1. PROWAZEK, Arch. f. Protistenk. 10 (1907); Centralbl. f. Bakt. 56 (1910) 41; Handbuch der pathogenen Protozoen, Leipsic (1912); Arch. f. Schiffs- u. Trop.-Hyg. 15 (1911) 173.

2. LIPSCHÜTZ, in Prowasek's Handbuch der pathogenen Protozoen, Leipsic (1912); Central f. Bakt. etc. I. Abt. Orig. **46** (1908) 609; Arch. f. Derm. u. Syph. **107** (1911) 387; **127** (1919–20) 193; **136** (1921) 428; Centralbl. f. Bakt. **87** (1921–22) 303.
   LIPSCHÜTZ and KUNDRATITZ, Wien. klin. Woch. **38** (1925) 499.
3. FINDLAY and LUDFORD, Brit. Journ. Exp. Path. **7** (1926) 223.
4. GOODPASTURE, Am. Journ. Path. **1** (1925) 1, 11, 29, 47, 547.
   GOODPASTURE and TEAGUE, Journ. Med. Res. **44** (1923–24) 121, 139, 185.
   GOODPASTURE and TALBOT, Am. Journ. Dis. Child. **21** (1921) 415.
5. SMITH, Ann. Missouri Bot. Gard. **13** (1926) 425.
6. RENAUT, Ann. de Derm. et de Syph. **2** (1881) 1.
7. GUARNIERI, Arch. per le Scienze med. **16** (1892) 403.
8. COUNCILMAN, MAGRATH, and BRINCKERHOFF, Journ. Med. Res. **11** (1904) 12.
9. CALKINS, Journ. Med. Res. **11** (1904) 136.
10. COPEMAN and MANN, 28th Ann. Rep. Local Govt. Board, London (1898) 505.
11. EWING, Journ. Med. Res. **12** (1904) 508; **13**: 233.
12. BORREL, Ann. de l'Inst. Past. **17** (1903) 81.
13. BÖING, Arb. a. d. Reichsgesundheitsamte **52** (1920) 615.
14. WOODCOCK, Journ. Roy. Army Med. Corps **37** (1921) 418.
15. COWDRY, Journ. Exp. Med. **36** (1922) 667.
    COWDRY and NICHOLSON, Journ. Exp. Med. **38** (1923) 695.
16. SCHÜTZ, Zeit. f. Hyg. u. Infekt. **105** (1925) 1.
17. PARKER and NYE, Am. Journ. Path. **1** (1925) 325, 337.
18. CRACIUM and OPPENHEIMER, Journ. Exp. Med. **43** (1926) 815.
19. BOSC, Centralbl. f. Bakt. **34** (1903) 413; Rev. gén. **4** (1904) 273.
20. PASCHEN, Münch. med. Woch. **55** (1908) 2494; **56** (1909) 2004.
21. GINS and RICKERT, Quoted from Hutyra and Marek 3d Am. ed. (1926).
22. BOLLINGER, Virch. Arch. **58** (1873) 349.
23. RIVOLTA, Ref. in Rivolta e Delprato's "L'ornitojatria," Pisa (1881).
24. MARX and STICKER, D. m. W. (1902) 893; (1903) 79.
25. MICHAELIS, Zeit. f. Krebsforsch. **1** (1903) 105.
26. LOEWENTHAL, Zeit. f. Krebsforsch. **5** (1907) 197.
27. LUGER and LAUDA, Wien. klin. Woch. **34** (1921) 132.
    LAUDA, Centralbl. f. Bakt. **91** (1923–24) 159.
28. DA FANO, Journ. Path. and Bact. **26** (1923) 85; **27** (1924) 11, 333.
    DA FANO and INGLEBY, Journ. Path. and Bact. **27** (1924) 349.
    DA FANO, Medical Science **10** (1924) 355.
29. LEVADITI, Ectodermoses Neurotropes, Paris (1922).
    LEVADITI, HARVIER, and NICOLAU, C. R. Soc. de biol. **84** (1921) 817; Ann. de l'Inst. Pasteur **36** (1922) 63.
    LEVADITI and HARVIER, Ann. de l'Inst. Pasteur **34** (1920) 911.
30. JOEST and DEGEN, Zeit. f. Infkr. **6** (1909) 348; **9**: 1; **10** (1911) 293.
31. NICOLAU and GALLOWAY, Brit. Journ. Exp. Path. **8** (1927) 336.
32. TYZZER, Journ. Med. Res. **14** (1905–06) 361; Philip. Journ. Sci. **1** (1906) 349.
33. BERTARELLI, Centralbl. f. Bakt. **50** (1909) 181.
34. KEYSSELITZ and MAYER, Arch. f. Protistenkunde **14** (1909) 113.

35. GINS, Zeit. f. Hyg. u. Infekt. **86** (1918) 299; Centralbl. f. Bakt. **88** (1922) 265.
36. RIVERS, Journ. Exp. Med. **43** (1926) 275.
37. RIVERS and TILLETT, Journ. Exp. Med. **38** (1923) 673.
38. ANDREWS and MILLER, Journ. Exp. Med. **40** (1924) 789.
39. WILE and KINGERY, Journ. Cutaneous Dis. **37** (1919) 431.
KINREGY, Arch. Dermatol. and Syph. **2** (1920) 144.
40. MACCALLUM, Journ. Cut. and Urin. Dis. **10** (1892) 93.
41. NEGRI, Bell. della Soc. med.-chir. di Pavia **1** (1903) 88; Zeit. f. Hyg. u. Infekt. **43**: 507
42. NOGUCHI, Journ. Exp. Med. **17** (1913) 29.
43. VOLPINO, Quoted from Hutyra and Marek, Am. ed. (1926) 564.
44. MANOUÉLIAN and VIALA, Ann. de l'Inst. Pasteur **38** (1924) 258.
45. ACTON and HARVEY, Parasitol. **4** (1911) 255.
46. MALLORY, Journ. Med. Res. **10** (1904–05) 483.
47. DÖHLE, Centralbl. f. Bakt. **61** (1912) 63.
48. MACEWEN, Journ. Path. and Bact. **18** (1913–14) 456.
49. HOEFER, Deutsch. med. Woch. **37** (1911) 1053.
50. SMIRNOWA-ZAMKOWA, Virchow's Arch. f. path. Anat. **261** (1926) 821.
51. HALBERSTÄDTER and PROWAZEK, Deutsch. med. Woch. **33** (1907) 1285. Berl. klin. Woch. **46** (1909) 1110.
52. NOGUCHI and COHEN, Arch. for Ophthal. **40** (1911) 1.
53. SOLOVIEF, Arch. des Inst. Past. de l'Afrique du Nord. **1** (1921) 388.
54. HERZOG, Arch. f. Ophth. **74** (1910) 520.
55. WILLIAMS, Arch. f. Ophth. **42** (1913) 506.
56. NOGUCHI, Journ. Exp. Med. **18** (1913) 572; Journ. Am. Med. Assoc. **89** (1927) 739.
57. LINDER, Wien. klin. Woch. (1909) 1555, 1659; Centralbl. f. Bakt. **55** (1910) 429; v. Graefe's Arch. f. Ophthal. (1913) 84.
58. WOLFRUM, 36te Versammlung d. ophthal. Ges. Heidelberg (1910) 207.
59. LIPSCHÜTZ, Wien. klin. Woch. **37** (1924) 286.
60. ULLMANN, Acta Oto-Laryngologica **5** (1923–24) 317.
61. WILE and KINGERY, Journ. Am. Med. Assoc. **73** (1919) 970.
62. JESIONEK and KIOLEMENOGLOU, Münch. med. Woch. **51** (1904) 1905.
63. RIBBERT, Centralbl. f. allg. Path. **15** (1904) 945.
64. LOEWENSTEIN, Centralbl. f. allg. Path. **18** (1907) 513.
65. PISANO, Gazz. d. osp. Milano **31** (1910) 249.
66. MOUCHET, Arch. de méd. expér. et d'anat. path. **23** (1911) 115.
67. SMITH and WEIDMAN, Univ. Penn. Med. Bull. **23** (1910) 285; Am. Journ. Trop. Dis. & Prev. Med. **2** (1914) 256.
68. DE LANGE, Virch. Arch. **227** (1922) 276.
69. MÜLLER, Virch. Arch. **238** (1922) 481.
70. VON GLAHN and PAPPENHEIMER, Am. Journ. Path. **1** (1925) 445 (lit.).
71. TERNI, D. t. W. (1908) 747.
72. RUHLE, Archiv f. Tierheilkunde **54** (1926) 197.
73. UHLENHUTH and BÖING, Berl. klin. Woch. **47** (1910) 1514.
74. ROSENTHAL, Centralbl. f. Bakt. **40** (1905) 204.
75. KLEINER, Zeit. f. Hyg. u. Infekt. **51** (1905–06) 177.
76. SCHIFFMAN, Wien. klin. Woch. **19** (1906) 1347.

77. PROWAZEK, M. m. W. (1908) 165, 1016.
78. MACFIE, Ann. Trop. Med. and Parasitol. 8 (1914) 439.
79. ADLER, Ann Trop. Med. and Parasitol. 19 (1925) 127.
80. KUHN, Centralbl. f. Bakt. 50 (1911) 31.
81. JACKSON, Journ. Infect. Dis. 26 (1920) 347.
82. COLE and KUTTNER, Proc. Soc. Exp. Biol. and Med. 23 (1926) 537.
83. WEISSENBERG, Handbuch der Pathogenen Protozoen (1921) No. 9, p. 1344 (lit.).
84. KEYSSELITZ, Arch. Protistenkunde 11 (1908) 326.
85. WHITE, U. S. Dept. Agr. Bull. 431 (1917) (lit.).
86. GLASSER, Journ. Agr. Res. 4 (1915) 121 (lit.); Science. N. S. 48 (1918) 301.
87. PAILLOT, Ann. de l'Inst. Pasteur 40 (1926) 314.
88. MATZ, Journ. Porto Rico Dept. Agr. 3 (1919) 65.
89. KUNKEL, Haw. Sugar Planters' Assoc. Exp. Sta. Bull. 3 (1921) 1.
90. PALM, Deli Proefsta., Medan, Sumatra Bull. 15 (1922) 1.
91. GOLDSTEIN, Bul. Torrey Bot. Club 51 (1924) 261.
92. RAWLINS and JOHNSON, Am. Journ. Bot. 12 (1925) 19.
93. MCKINNEY, WEBB, and ECKERSON, Journ. Agr. Res. 26 (1923) 605.
94. SMITH, Ann. Bot 38 (1924) 385
95. NELSON, Mich. Agr. Exp. Sta. Tech. Bull. 58 (1922) 1–30.
96. DUGGAR and KARRER, Phytopath. (1923) 13.

ACKNOWLEDGMENTS

For material and suggestions in the compilation of this work I am indebted to a great number of friends. I am especially grateful to Major Paul A. Schule who kindly read and corrected the manuscript and has offered many valuable suggestions. To Colonel E. B. Vedder and Major Raymond Kelser I am also indebted for many suggestions concerning various sections of the manuscript; to Dr. G. F. White for material he kindly sent to me regarding the disease of insects; to the various publishers for the use of illustrations. I am especially grateful for the advice and counsel of Dr. Frederick P. Gay and Dr. Frederick G. Novy concerning the publication of the manuscript and for suggestions as to the arrangement of the material. I appreciate to no small extent the facilities of the Bureau of Science, Manila, which were given me by Dr. William H. Brown, director. To Mrs. E. S. Basaca for her accurate typing of the manuscript and other assistance I am deeply grateful. The library staff of the Bureau of Science, Manila, deserves special mention for their help in bringing together a large part of the reference material. To all other friends who have given me their criticism of this work I extend, in this way, my deep appreciation.

ILLUSTRATIONS

PLATE 52

Bacteriophage lytic for *B. coli* showing lytic plaques on plates and in tubes. Note small number of plaques in dilution of 1:100,000 as compared to dilution of 1:10,000. The tubes represent successive dilutions of the lytic principle from 1:10 to 1:100,000.

PLATE 53

FIG. 1. Epithelial cells from rabbit's cornea, showing Guarnieri bodies after inoculation of cornea with vaccine bodies. (After Weissenberg.)

2. Pigeon pox. Inclusion bodies, "chicken pox bodies," in epithelial cells. (After Lipschütz; from Hutyra and Marek.)

3. Pox bodies and Guarnieri bodies; *a*, Paschen's pox bodies in epithelial cells from sections of a pox pustule; *b*, young Guarnieri bodies with central chromatin and peripheral plastin, in the cell plasma, at the side of the nucleus, an initial body; *c*, Guarnieri bodies with little chromatin and much plastin, pox bodies in the plasma of the cell; *d*, developed typical Guarnieri body, and initial body in the plastin; *b* and *d* are epithelial cells from an inoculated cornea of a rabbit. (After Hallenberger; from Hutyra and Marek.)

PLATE 54

FIG. 1. Intracellular bodies of mosaic-free plants; slime bodies in the sieve tubes of *Robinia pseudoacacia*. (After Strasburger.)

2. Bodies found in the sieve tubes of mosaic-free navy-bean plants.

3. Bodies found in the phloëm tissue of mosaic-free tomato plants. (After Doolittle and McKinney.)

PLATE 55

Vaccinia bodies. (After Findlay and Ludford.)

PLATE 56

Vaccinia bodies in corneal cells. (After Findlay and Ludford.)

PLATE 57

Vaccinia bodies (Cowdry). (After Findlay and Ludford.)

PLATE 58

Guarnieri bodies (Schütz). (After Findlay and Ludford.)

PLATE 59

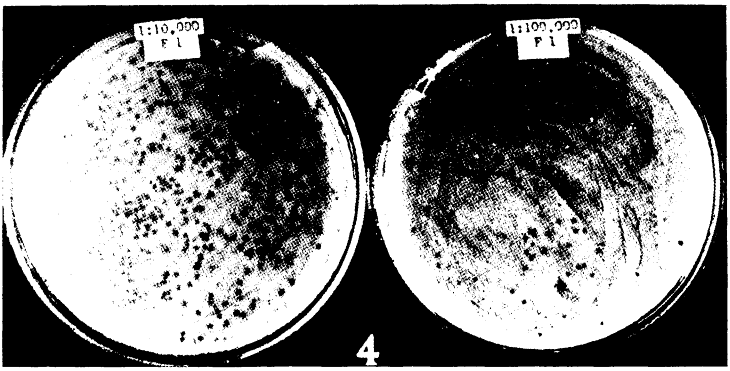
Inclusion bodies in various diseases. (After Findlay and Ludford.)

PLATE 60

Inclusion bodies in various diseases. (After Findlay and Ludford.)

PLATE 61

Negri bodies. (After Findlay and Ludford.)



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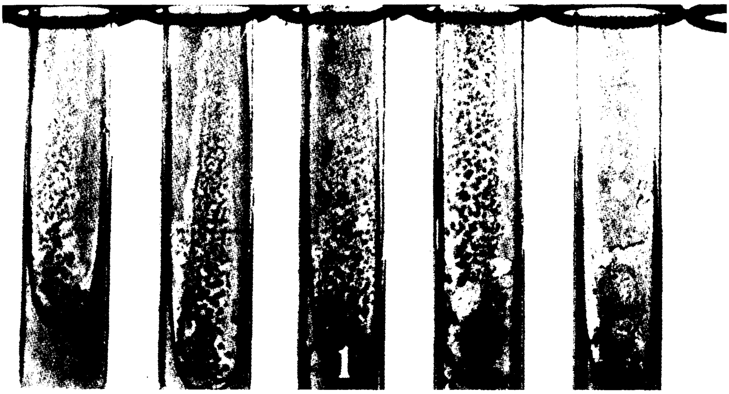

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PLATE 52. BACTERIOPHAGE LYTIC FOR B. COLI SHOWING LYTIC PLAQUES ON PLATES AND IN TUBES.

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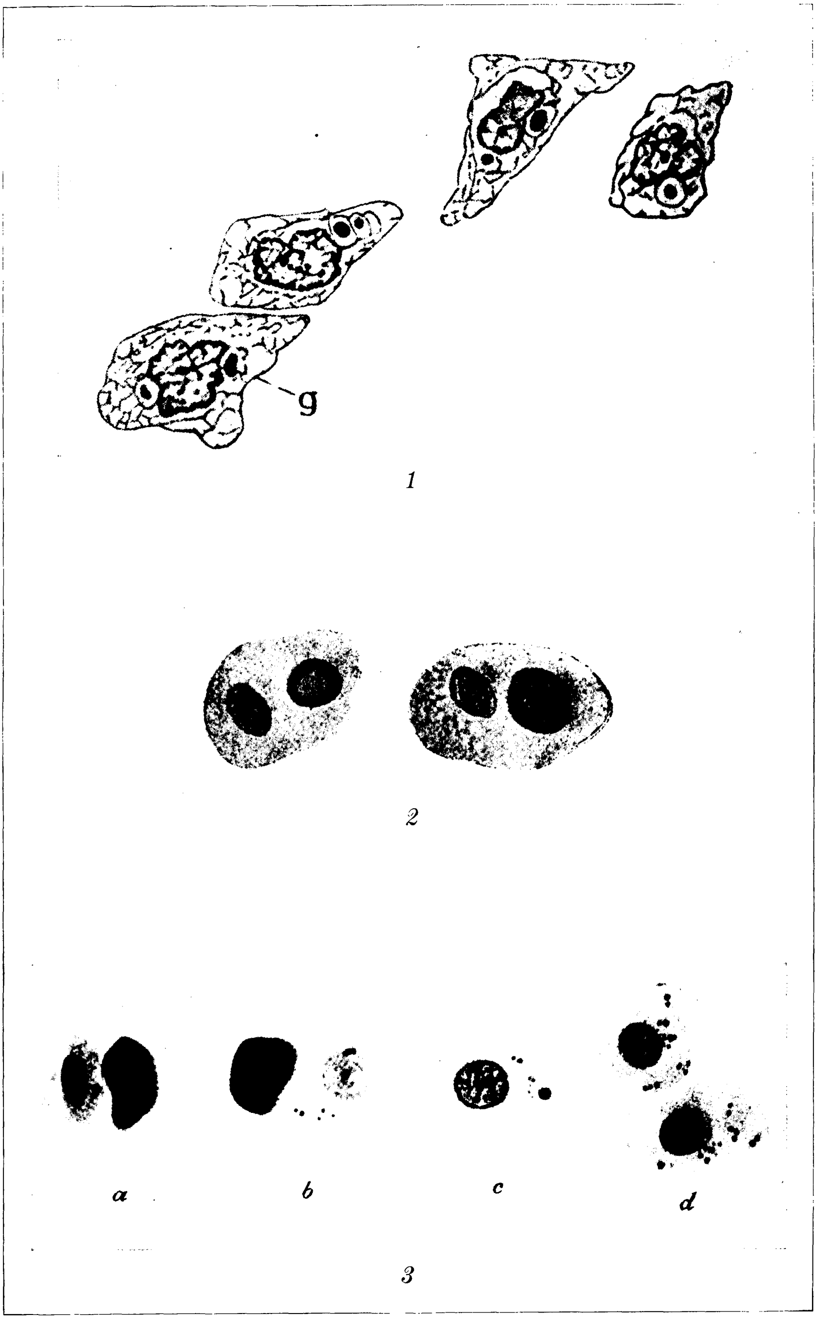

Fig. 1. Epithelial cells from rabbit's cornea showing Guarnieri bodies. 2. Pigeon-pox inclusion bodies. 3. Pox bodies and Guarnieri bodies.

PLATE 53.

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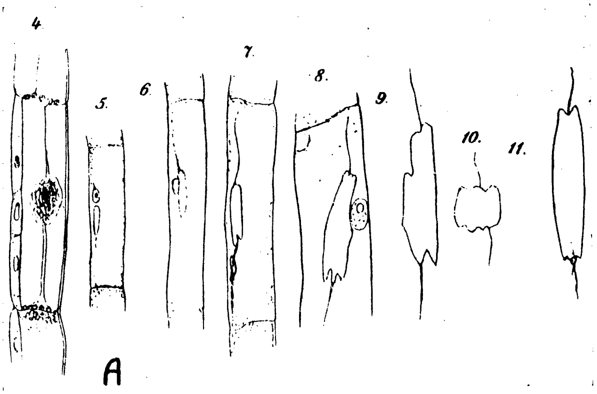

1

2

3

PLATE 54. INTRACELLULAR BODIES OF MOSAIC-FREE PLANTS.

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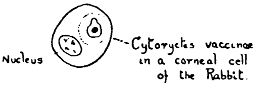
Cytoryctes vaccinae fixed alcohol sublimate stained carmine.



GUARNIERI Archivio per le Scienze mediche. 1892 16. 403

FIG. 1. [½]

—Vaccinia bodies in epidermal cells of Calf

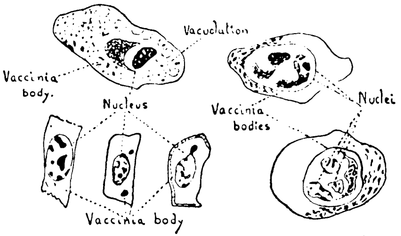


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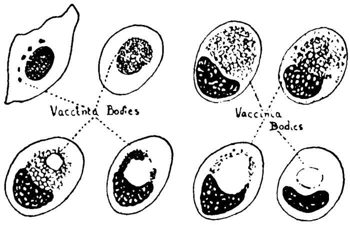
FIG 2 [½]



The Evolution of Vaccinia bodies in the Rats Cornea. Klatsch method Nocht-Romanowsky stain.

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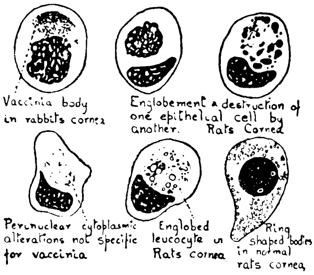
FIG 3 [½]


Vaccinia body in rabbits cornea
Englobement a destruction of one epithelial cell by another. Rats Cornea
Perinuclear cytoplasmic alterations not specific for vaccinia
Englobed leucocyte in Rats cornea
Ring shaped bodies in normal rats cornea

Conditions simulating Vaccinia bodies in Corneal Cells Klatsch method

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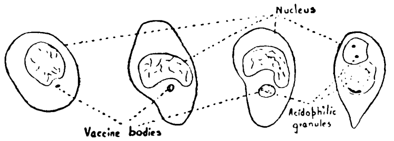
FIG. 4. [½]

Development of Vaccine bodies in the Corneal cells of the Rabbit fixed Zenker stained Azur I



BOING Arbeiten aus dem Reichgesundheitsamte 1920 52 615

FIG. 5 [½]

PLATE 55. VACCINIA BODIES.

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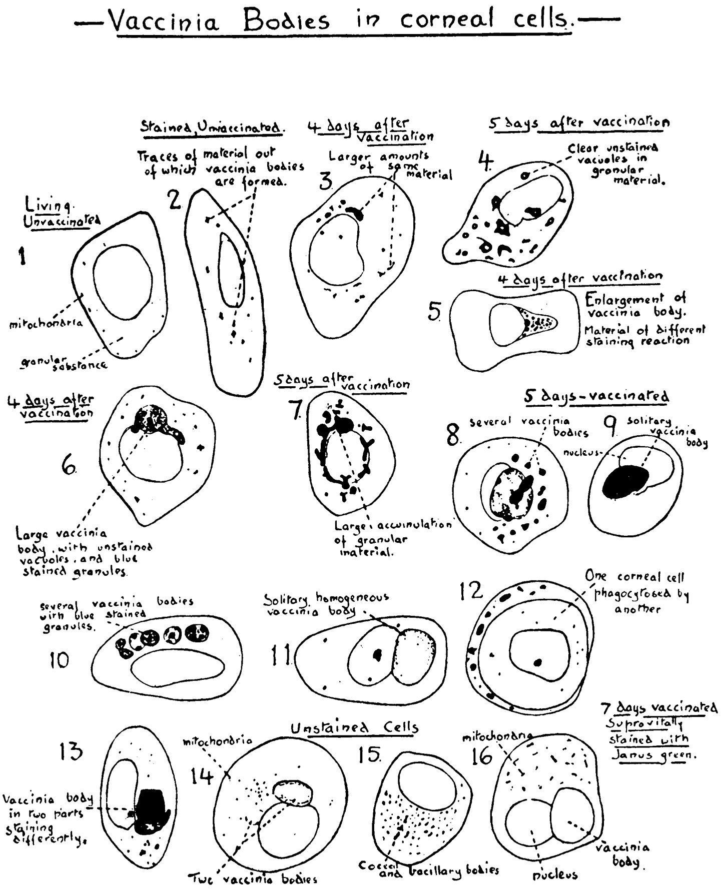
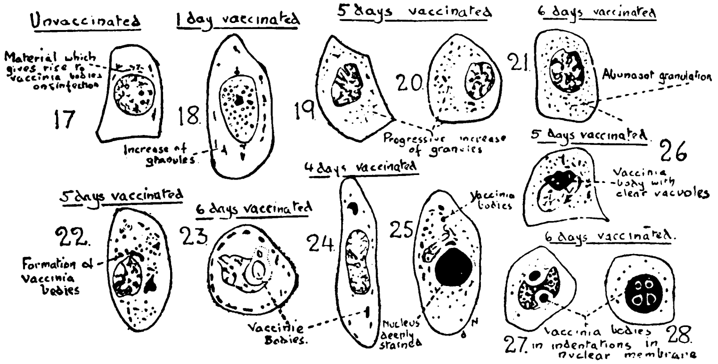

—Vaccinia Bodies in corneal cells.—
Living. Unvaccinated
1
mitochondria
granular substance
Stained, Unvaccinated.
Traces of material out of which vaccinia bodies are formed.
2
4 days after vaccination
Larger amounts of same material
3
5 days after vaccination
4
Clear unstained vacuoles in granular material.
4 days after vaccination
5
Enlargement of vaccinia body.
Material of different staining reaction
4 days after vaccination
6
Large vaccinia body, with unstained vacuoles, and blue stained granules.
5 days after vaccination
7
Large accumulation of granular material.
5 days-vaccinated
8
Several vaccinia bodies
9
Solitary vaccinia body
nucleus
Several vaccinia bodies with blue stained granules.
10
Solitary homogeneous vaccinia body
11
12
One corneal cell phagocytosed by another
7 days vaccinated
Supravitally stained with Janus green.
Unstained Cells
13
Vaccinia body in two parts staining differently.
mitochondria
14
Two vaccinia bodies
15
Coccal and bacillary bodies
16
mitochondria
nucleus
vaccinia body.

FIG. 6. [2/3]

PLATE 56. VACCINIA BODIES IN CORNEAL CELLS.



Histological Technique Employed

No. of Fig.	Fixative.	Staining Method	No. of Fig.	Fixative	Staining Method.
1	none	none.	15	none	none
2	"	Stained intra vitam with brilliant cresyl blue 2B.	16	"	Janus green B.
3	"		17	Zenker.	Giemsa
4	"		18	Zenker without acetic acid	Giemsa
5	"		19		
6	"	"	20		
7	"	"	21	"	"
8	"	"	22	Zenker	Giemsa.
9	"	"	23	Zenker	Wright
10	"	"	24	Zenker	carbol-fuchsin and acid violet.
11	"	"	25	Acetic sublimate.	Giemsa
12	"	"	26	Zenker.	Giemsa.
13	"	"	27	Zenker	Giemsa
14	"	none	28	Giemsa sublimate	Giemsa.

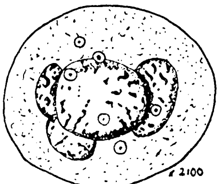
COWDRY, JOUR. EXPT. MED., 1922, VOL. XXXVI. 666

FIG. 7. [2/3]

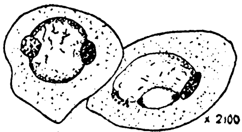
PLATE 57. VACCINIA BODIES (COWDRY).

UNIV. OF MICH.

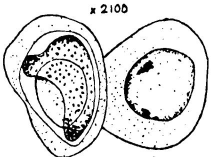
Stages in the development of Guarnieri bodies in the cornea & skin of the Rabbit: fixed Champy: stained eosin haematoxylin.



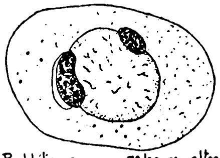
Rabbits cornea: giant cell · 24 hours after vaccination



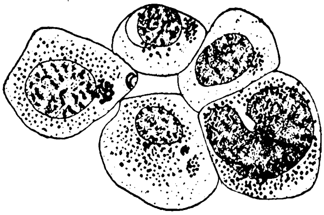
Rabbits cornea: 70 hours after vaccination · Guarnieri bodies at the cell poles



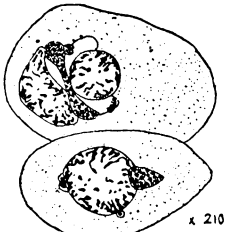
Rabbits cornea: 70 hours after vaccination



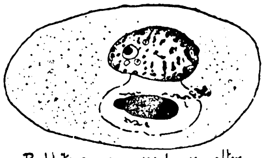
Rabbit's cornea 70 hours after vaccination x 2100



Disintegration of Guarnieri bodies in Rabbits skin 100 hours after vaccination. x 2100



Rabbits cornea 70 hours after vaccination



Rabbits cornea 100 hours after vaccination x 2100

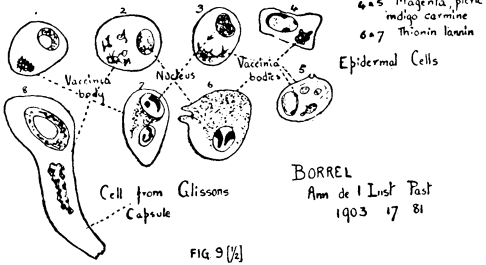
SCHUTZ Zeit f Hyg u Infekt. 1925-6 105 1

FIG. 8.[2/3]

PLATE 58. GUARNIERI BODIES (SCHÜTZ).

UNIV. OF MICH.

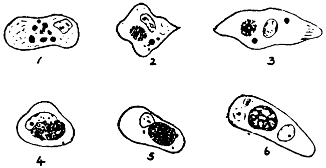
Cells of Sheep infected with Sheep pox stained 1 3 Laverans method
4 & 5 Magenta, picric indigo carmine
6 & 7 Thionin lannin



BORREL
Ann de l Inst Past
1903 17 81

FIG. 9 [½]

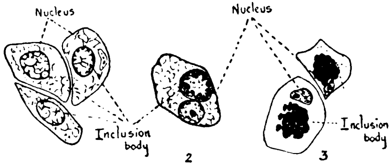
Cytoplasmic inclusions in epidermal cells of Hen with Fowl pox
fixed Flemming's solution stained 1·3 Safranin Indigo carmine
Picric Acid 4·6 Gentian violet, Vesuvin



APOLANT Virchow's Archiv f path Anat. 1903 174. 86.

FIG. 10 [½]

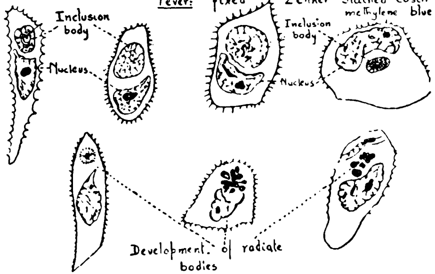
Cytoplasmic inclusions in epidermal cells of Pigeon with Fowl pox
fixed formalin: stained 1 Iron haematoxylin and Orange G 2 Alum haematoxylin and Orange G 3 Iron haematoxylin van Gieson



MICHAELIS Zeitschrift f. Krebsforschung 1903. 1 105.

FIG. 11. [½]

Inclusion bodies from epidermal cells in Scarlet Fever: fixed Zenker stained eosin methylene blue



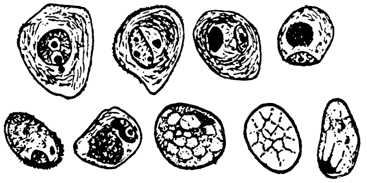
MALLORY. J. Med. Res. 1903·4. 10. 483

FIG. 12 [½]

PLATE 59. INCLUSION BODIES IN VARIOUS DISEASES.

UNIV. OF MICH.

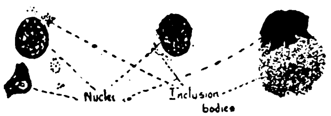
Molluscum contagiosum in the epidermal cells of Man fixed in alcohol or corrosive sublimate stained Delafields hoematoxylin and eosin



A. B MACALLUM J. Cutaneous a Genito Urin Dis 1892 10 93

FIG. 13. [½]

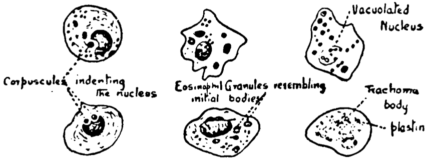
Stages in the development of Inclusion bodies in Trachoma in the cells of the Conjunctival Epithelium stained Giemsa



NOGUCHI and COHEN J Exp Med. 1913 18 572

FIG. 14. [½]

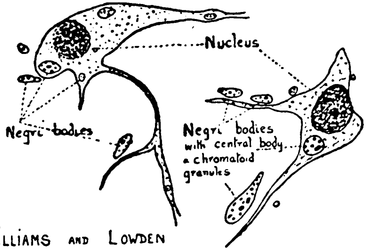
Epithelial cells of the conjunctiva from Trachoma in Man. fixed Schaudinn stained Giemsa


Vacuolated Nucleus
Corpuscules indenting the nucleus
Eosinophil Granules resembling initial bodies
Trachoma body
plastin

SOLOVIEF Archives des Inst Past de l Afrique du Nord 1921, 1 388.

FIG. 15 [½]

Negri bodies in cells of Ammon's horn of Dog with Rabies

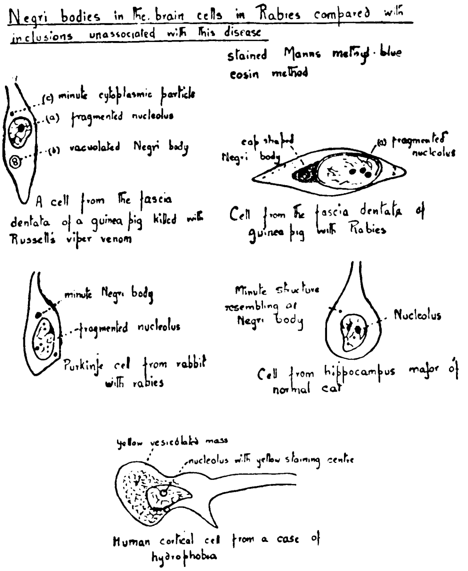


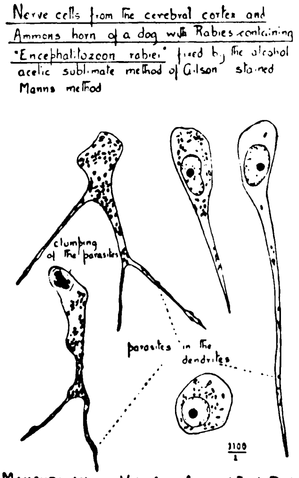
WILLIAMS AND LOWDEN J. Infect. Dis 1906 3 452

FIG. 16. [½]

PLATE 60. INCLUSION BODIES IN VARIOUS DISEASES.

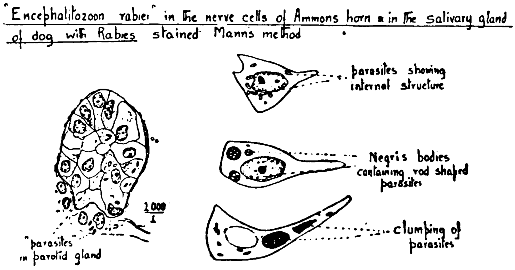
UNIV. OF MICH.


Negri bodies in the brain cells in Rabies compared with inclusions unassociated with this disease
stained Manns method - blue eosin method
(c) minute cytoplasmic particle
(a) fragmented nucleolus
(b) vacuolated Negri body
A cell from the fascia dentata of a guinea pig killed with Russell's viper venom
cap shaped Negri body
(a) fragmented nucleolus
Cell from the fascia dentata of guinea pig with Rabies
minute Negri body
fragmented nucleolus
Purkinje cel from rabbit with rabies
Minute structure resembling a Negri body
Nucleolus
Cell from hippocampus major of normal cat
yellow vesicolated mass
nucleolus with yellow staining centre
Human cortical cel from a case of hydrophobia


Nerve cells from the cerebral cortex and Ammons horn of a dog with Rabies containing "Encephalitozoon rabiei" fixed by the alcohol acetic sublimate method of Gilson stained Manns method
clumping of the parasites
parasites in the dendrites
1100/1

MANOUELIAN and VIALA Ann de l Inst Past 1924 38 258
FIG. 18.[½]

ACTON and HARVEY Parasitol. 1911 4 255
FIG. 17.[½]

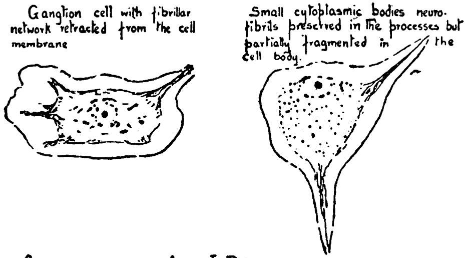

"Encephalitozoon rabiei" in the nerve cells of Ammons horn & in the salivary gland of dog with Rabies stained Manns method
parasites showing internal structure
Negris bodies containing rod shaped parasites
clumping of parasites
1000/1
"parasites" in parotid gland

MANOUELIAN and VIALA Ann. de l Inst Past 1924 38 258
FIG. 19.[½]

PLATE 61. NEGRI BODIES.

UNIV. OF MICH.

Stages in the development of Negri bodies in the brain of the Rabbit stained by carbol anilin fuchsin

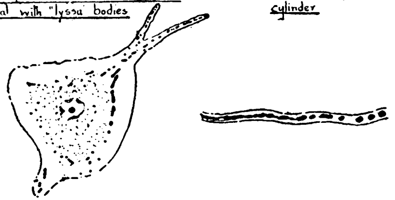

Ganglion cell with fibrillar network retracted from the cell membrane
Small cytoplasmic bodies neuro-fibrils preserved in the processes but partially fragmented in the cell body.

GOODPASTURE. Amer J Path 1925. 1. 547

FIG. 20 [½]

Ganglion cell showing final break up of the neurofibrillar material into bodies identical with "lyssa" bodies

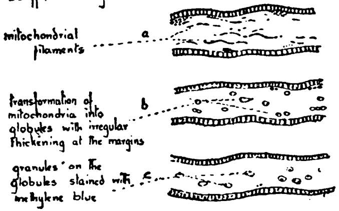
Formation of hyaline bodies in an axis cylinder



GOODPASTURE Amer. J Path 1925 1 547

FIG. 21. [½]

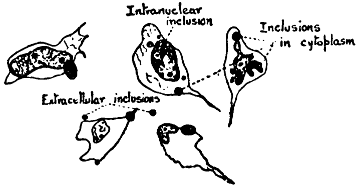
Stages in the development of Negri bodies in the brain of the Rabbit fixed Zenker stained a & b fuchsin, methyl green: c acid fuchsin & Loefflers methylene blue


mitochondrial filaments
a
transformation of mitochondria into globules with irregular thickening at the margins
b
granules on the globules stained with methylene blue
c

GOODPASTURE Amer. J Path. 1925. 1 547

FIG. 22. [½]

Inclusion bodies in ganglion cells of Ammon's horn of dog with Distemper stained eosin-methylene blue



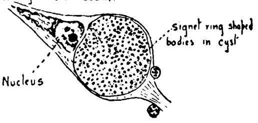
LENTZ Zeitschr. f. Hygiene u. Infect. 1909 62. 63.

FIG. 23. [½].

PLATE 62. STAGES IN THE DEVELOPMENT OF NEGRI BODIES, "LYSSA" BODIES, AND INCLUSION BODIES IN DISTEMPER.

UNIV. OF MICH.

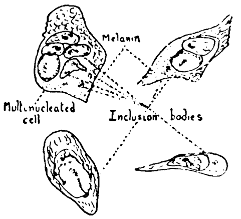
Ganglion cell from brain of dog with distemper containing a cyst stained Heidenhain's haematoxylin and eosin.



KANTOROWICZ and LEWY Archiv f Wiss u prak Tierheilkun 1922·3. 49 137

FIG. 24. [½]

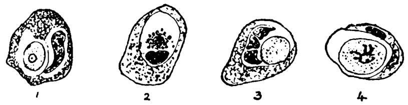
Epidermal cells from Varicella in Man: fixed in Zenker stained eosin methylene blue



TYZZER. J. Med Res 1905·6 14 361.

FIG. 25. [½]

Varicella inclusion bodies in the epidermal cells of the Rabbit.

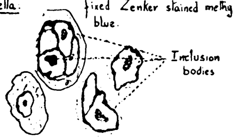


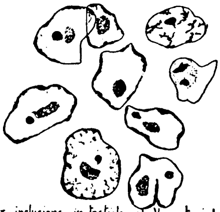
fixed Alcohol sublimate : stained 1, 3 and 4 Giemsa. 2 Mansons stain
1 Inclusion in almost normal epidermal cell
2 Disintegrating inclusion body
3 Giant cell with inclusion body.
4 Inclusion body with internal granules.

GINS Zeitschrift f Hygiene 1915 86. 299

FIG. 26. [½]

Nuclear inclusions in epidermal cells of man. Varicella. fixed Zenker stained methylene blue.





Nuclear inclusions in testicle of Vervet injected with human varicella material

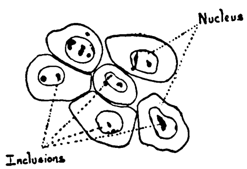
RIVERS J. Exp. Med 1926 43 275.

FIG. 27. [½]

PLATE 63. INCLUSION BODIES IN DISTEMPER AND VARICELLA.

UNIV. OF MICH.

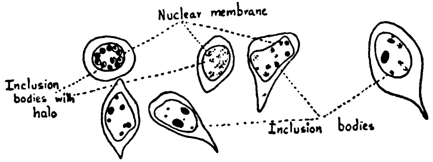
Herpetic Inclusions
stained Giemsa



LIPSCHUTZ Archiv. f. Dermat. u Syph. 1921. 136. 428.

FIG. 28. [½]

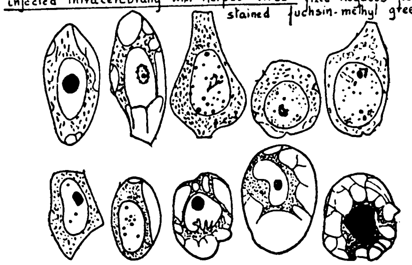
Inclusion bodies from Nerve Cells of Rabbit's brain infected with Encephalitis lethargica stained Mann's method



LEVADITI "Poliomyelite et Encephalite 1922 Paris.
Ectodermoses neurotropes, a Ann de l'Inst. Past. 1922 36 77.

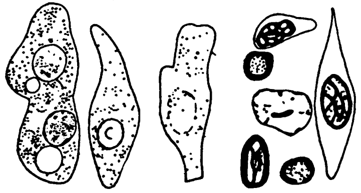
FIG. 29. [½]

'Nuclear inclusions in nerve cells of nucleus caudatus of Rabbit injected Intracerebrally with Herpes virus. fixed Regauds fluid; stained fuchsin-methyl green.



COWDRY AND F. M. NICHOLSON J. Exp. Med. 1923 38. 695.

Cells and isolated nuclei from brain of Rabbit injected intracerebrally with Herpetic virus: air dried smears stained Giemsa: dark blue granules in both cells and some nuclei



COWDRY AND F. M. NICHOLSON. J. Exp. Med. 1923. 38. 695.

PLATE 64. HERPETIC INCLUSIONS.

UNIV. OF MICH.

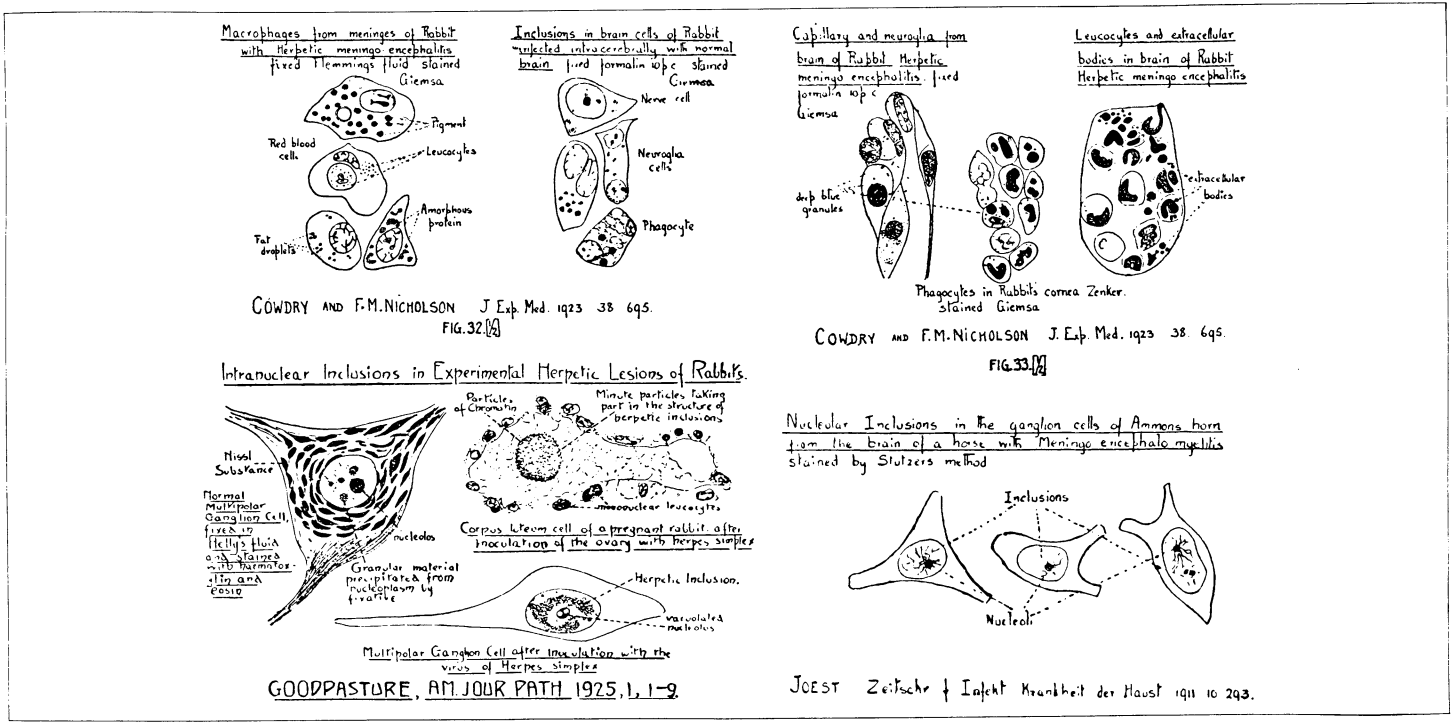

Macrophages from meninges of Rabbit with Herpetic meningo encephalitis fixed Flemmings fluid stained Giemsa
Pigment
Red blood cells
Leucocytes
Amorphous protein
Fat droplets
Inclusions in brain cells of Rabbit injected intracerebrally with normal brain fixed formalin 10 p c stained Giemsa
Nerve cell
Neuroglia cells
Phagocyte
COWDRY AND F.M.NICHOLSON J Exp. Med. 1923 38 695.
FIG. 32.[½]
Capillary and neuroglia from brain of Rabbit Herpetic meningo encephalitis. fixed formalin 10 p c Giemsa
deep blue granules
Phagocytes in Rabbits cornea Zenker. stained Giemsa
Leucocytes and extracellular bodies in brain of Rabbit Herpetic meningo encephalitis
extracellular bodies
COWDRY AND F.M.NICHOLSON J. Exp. Med. 1923 38. 695.
FIG.33.[½]
Intranuclear Inclusions in Experimental Herpetic Lesions of Rabbits.
Nissl Substance
Normal Multipolar Ganglion Cell, fixed in Hellys fluid and stained with haematoxylin and eosin
nucleolus
Granular material precipitated from nucleoplasm by fixative
Particles of Chromatin
Minute particles taking part in the structure of herpetic inclusions
mononuclear leucocytes
Corpus luteum cell of a pregnant rabbit. after inoculation of the ovary with herpes simplex
Herpetic Inclusion.
vacuolated nucleolus
Multipolar Ganglion Cell after inoculation with the virus of Herpes simplex
GOODPASTURE, AM. JOUR PATH 1925, 1, 1–9.
Nucleolar Inclusions in the ganglion cells of Ammons horn from the brain of a horse with Meningo encephalo myelitis stained by Stutzers method
Inclusions
Nucleoli
JOEST Zeitschr f Infekt Krankheit der Haust 1911 10 293.

PLATE 65. HERPETIC INCLUSIONS AND INCLUSION BODIES IN BORNA DISEASE.

UNIV. OF MICH.

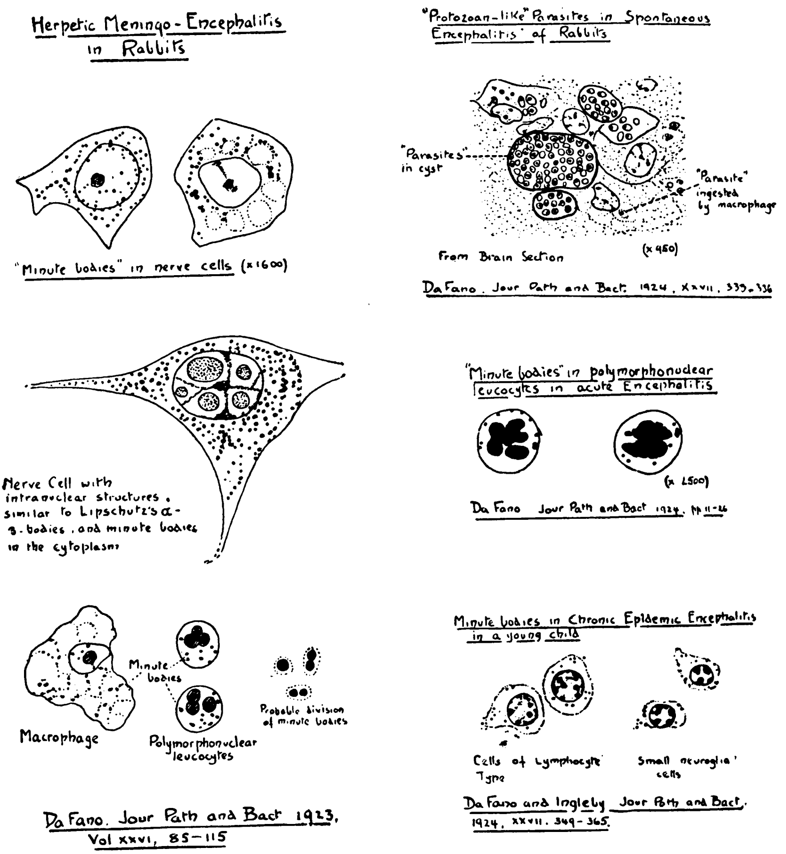
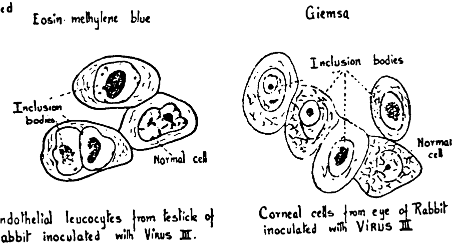

Herpetic Meningo-Encephalitis in Rabbits
"Minute bodies" in nerve cells (x1600)
Nerve Cell with intranuclear structures, similar to Lipschutz's α-β-bodies, and minute bodies in the cytoplasm
Macrophage
Minute bodies
Polymorphonuclear leucocytes
Probable division of minute bodies
Da Fano. Jour Path and Bact 1923, Vol xxvi, 85-115
"Protozoan-like" Parasites in Spontaneous Encephalitis' of Rabbits
"Parasites" in cyst
"Parasite" ingested by macrophage
From Brain Section
(x 450)
Da Fano. Jour Path and Bact. 1924, xxvii, 333-336
"Minute bodies" in polymorphonuclear leucocytes in acute Encephalitis
(x 1500)
Da Fano Jour Path and Bact 1924, pp 11-26
Minute bodies in Chronic Epidemic Encephalitis in a young child
Cells of Lymphocyte Type
Small neuroglia' cells
Da Fano and Ingleby Jour Path and Bact, 1924, xxvii, 349-365.

FIG. 34 [2/3]

PLATE 66. HERPETIC INCLUSIONS, "PROTOZOAN-LIKE" PARASITES IN SPONTANEOUS ENCEPHALITIS OF RABBITS, BODIES IN ACUTE ENCEPHALITIS AND CHRONIC EPIDEMIC ENCEPHALITIS.

UNIV. OF MICH.

Nuclear inclusions in cells of Rabbit inoculated with Virus III fixed Zenker stained



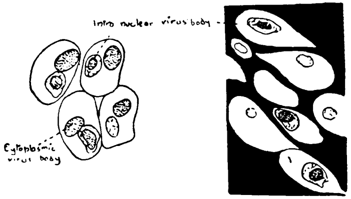
Endothelial leucocytes from testicle of Rabbit inoculated with Virus III.

Corneal cells from eye of Rabbit inoculated with VIRUS III

RIVERS AND TILLETT J. Exp. Med. 1924 40. 281.

FIG. 37. [½]

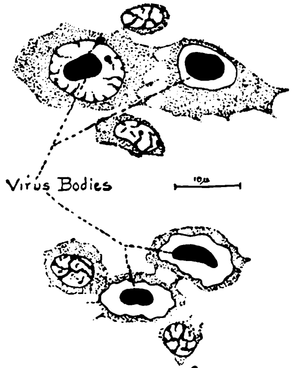
Cytoplasmic and Intranuclear Inclusions in Paravaccinia



LIPSCHUTZ, ARCH. DERMAT und SIPHILIS, 1919-20, 126

FIG. 39. [½]

Nuclear inclusions in cells of Rabbits testicle fixed Zenker stained eosin methylene blue. (drawn from a section lent by the authors)



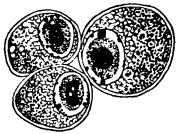
MILLER, ANDREWES and SWIFT J Exp Med. 1924 40 773 a 789

FIG. 38. [½]

PLATE 67. NUCLEAR INCLUSIONS IN CELLS OF RABBIT INOCULATED WITH VIRUS III; INCLUSION BODIES IN PARAVACCINIA.

UNIV. OF MICH.

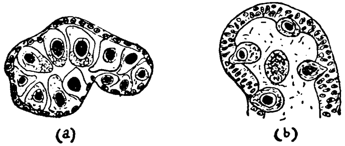
Nuclear inclusions in the renal epithelium of a syphilitic fœtus stained Hæmatoxylin and eosin



JESIONEK and KIOLEMENOGLOU Munch. med Woch 1904 51 1905

FIG. 40. [½]

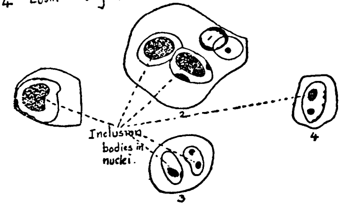
Intranuclear inclusions in (a) the Kidneys (b) the salivary glands of infants



RIBBERT Centralbl. f allgemeine path u path Anatomie 1904 15. 945.

FIG. 41. [½]

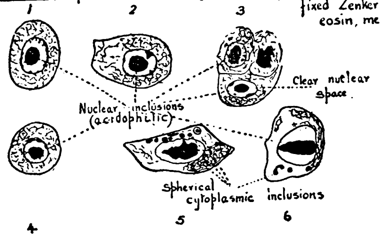
Intranuclear inclusions in the cells of the lung of an adult dying with obscure symptoms stained 1 Haematoxylin and eosin 2-4 Eosin methylene blue



VONGLAHN AND PAPPENHEIMER Amer. J Path 1925. 1. 445

FIG. 42. [½]

Cytoplasmic inclusions unassociated with any known virus infection
1-4 Cells from bronchial exudate of infant dying with broncho-pneumonia.
5 & 6 Cells in wall of serous salivary gland of Guinea pig
fixed Zenker stained eosin, methylene blue


1
2
3
Clear nuclear space.
Nuclear inclusions (acidophilic)
Spherical cytoplasmic inclusions
4
5
6

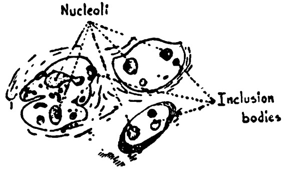
GOODPASTURE AND TALBOT Amer. J. Diseases of Children 1921 21. 415

FIG. 43. [½]

PLATE 68. VARIOUS INCLUSION BODIES.

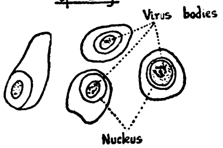
UNIV. OF MICH.

Inclusions in the epidermal cells of the tongue of the Guinea pig with Foot & Mouth disease · stained Giemsa



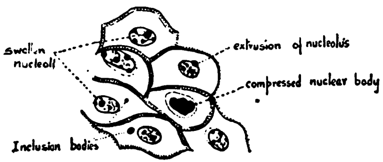
GINS. Centralbl f Bakt. 1922 88 265
FIG. 44. [½]

Cells from Infective Warts of Dog: stained Giemsa.



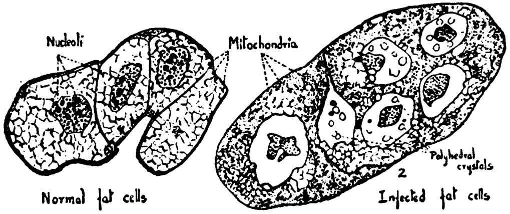
ULLMANN. Acta Medica Laryn. 1923-4. 5 317
FIG. 45. [½]

Epidermal cells from Verruca vulgaris - infective warts in Man.



KYRLE "Histo biologie der Haut" Berlin 1925
FIG. 46. [½]

Fat cells of the Caterpillar of the Silk Worm Moth (Bombys mori) infected with the polyhedral virus — "grasserie" fixed (1) in Regaud's sol. (2) in formol saline stained acid fuchsin, anilin and toluidin blue (Kull's method)

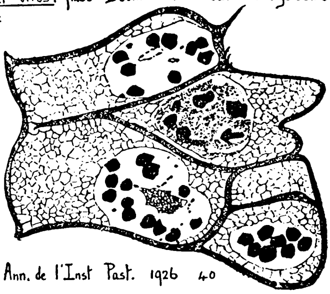


PAILLOT Ann de l Inst Past 1926 40 314
FIG. 47. [½]

PLATE 69. INCLUSION BODIES IN VARIOUS DISEASES.

UNIV. OF MICH.

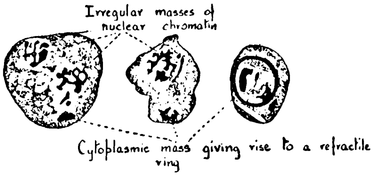
Fat cells of the Caterpillar of the Silk Worm Moth (Bombyx mori) infected with the polyhedral virus: fixed Bouin stained carmine hydrochloride picric indigo-carmine



PAILLOT Ann. de l'Inst Past. 1926 40 314

FIG.48 [1/2]

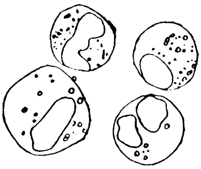
Micronucleocytes from the blood of the caterpillar of the large Cabbage White Butterfly (Pieris brassicae) inoculated with blood from a caterpillar infected with nuclear disease - maladie du noyau stained Giemsa.


Irregular masses of nuclear chromatin
Cytoplasmic mass giving rise to a refractile ring

PAILLOT Ann de l'Inst Past 1926 40. 314

FIG. 49. [1/2]

Inclusions in the leucocytes of fowls suffering from an acute infective disease stained Giemsa



MACFIE Annals of Trop Med and Parasitol 1914 8 439

FIG. 50. [1/2]

PLATE 70. INCLUSION BODIES IN DISEASES OF INSECTS AND FOWLS.

UNIV. OF MICH.

PLATE 62

Stages in the development of Negri bodies, "lyssa" bodies, and inclusion bodies in distemper. (After Findlay and Ludford.)

PLATE 63

Inclusion bodies in distemper and varicella. (After Findlay and Ludford.)

PLATE 64

Herpetic inclusions. (After Findlay and Ludford.)

PLATE 65

Herpetic inclusions and inclusion bodies in Borna disease. (After Findlay and Ludford.)

PLATE 66

Herpetic inclusions, "protozoan-like" parasites in spontaneous encephalitis of rabbits, bodies in acute encephalitis and chronic epidemic encephalitis. (After Findlay and Landford.)

PLATE 67

Nuclear inclusions in cells of rabbit inoculated with virus III; inclusion bodies in paravaccinia. (After Findlay and Ludford.)

PLATE 68

Various inclusion bodies. (After Findlay and Ludford.)

PLATE 69

Inclusion bodies in various diseases. (After Findlay and Ludford.)

PLATE 70

Inclusion bodies in diseases of insects and fowls. (After Findlay and Ludford.)

TEXT FIGURES

FIG. 1. Fever curve in dog distemper. Catarrh of the air passages, later gastroenteritis, toward the termination catarrhal pneumonia. (After Hutyra and Marek.)

2. Female phlebotomus, vector in pappataci fever; *a*, antenna; *b*, palps; *c*, probocis; *d*, prothorax; *e*, mesothorax; *f*, halteres. (After Alcock; from Manson.)

3. Fever curve in a typical case of foot-and-mouth disease. (From Hutyra and Marek.)

4. Artificial hog-cholera infection with filtered material from a hog affected with cholera. The first rise in temperature is caused by the primary infection, the second by the secondary infection. (After Hutyra and Marek.)

5. Case of trench fever. (After the Trench Fever Report of Commission Medical Research Committee, American Red Cross.)

6. Case of trench fever. (After the Trench Fever Report of Commission Medical Research Committee, American Red Cross.)

7. The minimal lethal exposures (abscissa) for bacteriophage in dilutions 1 : 10 to 1 : 10,000,000 in three broths, A, B, and C, plotted according to the logarithms of the dilutions (ordinate).

CHAPTER XVIII

REVIEW OF VIRUS DISEASES

The previous chapters have been devoted to a description of the various filterable virus and rickettsia diseases. An attempt has been made to present the facts concerning each disease, its etiologic agent, and the theories, speculations, and contemporary views pertaining to it. From the literature have been selected those works which seem to be most important. The material has been presented in a descriptive rather than analytical form in order that the review might be of most value to all types of students. This chapter deals in a more critical manner with some of the general aspects of the subject and presents the present status of this subject with the trend of future developments in the field.

THE COMPOUND AND ULTRAMICROSCOPE, LIMITS OF VISIBILITY AND SIZE OF ULTRAVIRUSES

The sense of sight imparts to us the greatest proportion of our knowledge, and all living things endowed with this sense have learned by experience to place confidence and reliance in what is learned by the eye. Normal vision in man is developed to a very high degree, although some animals are said to have a more highly developed sense of sight than man. The unaided normal human eye can detect a black thread 0.005 inch in diameter on a white background at a distance of about 100 feet, and the angle subtended is 0.55 second. For small objects which are invisible to the unaided eye man has devised and made use of instruments such as the telescope, the microscope, the dark-field microscope, and the ultramicroscope. Only when the number is large can these smaller objects be detected by the unaided eye. Bacteria are among the smallest of living cells and when massed together in considerable numbers forming a colony they become visible not as individuals but as a group of individuals. For example it has been estimated that about 2,000,000,000 cocci of 1 micron in diameter if grouped together would form a colony about the size of a small pinhead. This group if spread out side by side would present a surface of about 5,000 square millimeters. A colony of typhoid bacilli 1 millimeter in diameter contains approximately 500,000,000 germs. When such small objects are grouped in this way they readily become visible to the unaided eye. Bacteria were among the first microscopic objects to be seen with the aid of the simple microscope. In general it may be stated that the micrococci measure about 1 micron in diameter; the bacilli are about 1 micron in thickness and 3 microns in length; the spirilla are of about the same thickness and measure 6 microns in length. The spirochætes posses less thickness than bacilli and spirilla and are usually stated to measure about 0.1 by 8 microns. There are many variations from these measurements but those given will hold for a large number of these smaller cells. Some spirochætes are invisible with ordinary microscopes; such are *Spirochæta icteroides* and *S. icterohæmorrhagiæ* which are probably closely related if not identical organisms. For such spirochætes we make use of the dark-field by which they are readily brought into vision.

Since the first microscopes were invented much progress in their development has been made as the direct result of the demands of science. Amateur scientists interested in the markings and ornaments in the shells of the Diatomaceæ by their insistent demands for improved instruments have contributed largely to this progress. The improvements in the microscope, however, reached a point beyond which it is very difficult to extend. The results that can be obtained with the best of these instruments are limited. Cotton and Mouton (1) state:

> It is true that greater magnifying power might be attained, but this is without real interest, for the ideal microscope would be one in which each point in the object under view would be imaged forth by a point of inappreciable dimensions. But what really happens is this: Even in the best instruments, the image of a point is always a small spot whose dimensions increase with the increase of the magnifying power of the instrument, so that when two points are too near one another in the object under examination, their images lap over one another a little and the eye is no longer able to separate them. The smallest resolvable distance, though this depends somewhat on the nature of the object that is under examination, is never less than a quarter of a thousandth of a millimeter, even when the very best instruments are used. The explanation of this limitation of the powers of the microscope has been found in the nature of light itself. Considering light to be a result of vibratory motion and taking into account the phenomenon of diffraction which is peculiar to the microscope as to all optical instruments, we can calculate the diameter of the spot forming the image of a point and show that this diameter will be smaller if, in the first place, the cone of rays which the instrument receives from the point under consideration is more open, and, in the second place, if this point is placed in a medium of higher index and sends out rays of shorter wavelength, viz., nearer the violet in the spectrum, if we consider only visible

rays. But with the instruments now in use, permitting homogenous immersion and great numerical aperture, we may say that the limit assigned theoretically to the resolving power of the microscope has practically been reached. We may hope to extend it a little further by employing ultraviolet light, to which, though the eye is not sensitive, the photographic plate is sensitive. Yet even by this method, we can hardly hope to do more than double the resolving power of the apparatus and this only after surmounting great technical difficulties.

The general theory of microscope optics is well known. The image formed by a microscope objective is considered to be made up of a number of points. For the purpose of illustration we will take two of these points. The first point in question we will consider as the image of the point where the axis of the objective intersects the object. A bundle of rays proceed from this point and are brought to a focus in the image plane of the objective if the field lens of the eyepiece does not intervene. The field lens places this image in the plane of the diaphragm and the focal plane of the eye lens of the eyepiece. The eye lens forms an image of this point at infinity which may be observed by an eye completely at rest. A shift of the fine adjustment changes the whole optical system and the final image is not placed at infinity; in this case the eye is placed under a constant strain to compensate for the lack of proper focus of the instrument.

The second point to be considered is at the extreme edge of the field. The rays proceeding from this point are not symmetrical about a central ray as in the first instance. However, one ray of this bundle is the principal ray and it proceeds through the objective without angular deviation. The image point is the same and the field lens places the image in the same plane as in the first case but at the edge of the diaphragm. The eye lens of the eyepiece deviates the principal ray so that it crosses the axis at a point known as Ramsden's disc, or eyepoint, and it is at this point that the pupil of the eye should be placed to observe the entire field. The magnification of the objective is dependent on the distance of the optical center of the objective from the object and also from the image. The distance of the optical center of the objective from the image depends upon the tube length. Usually the magnification of all objectives is computed for a standardized tube length of 160 millimeters. The eyepiece magnifies the image formed by the objective on the principle of the simple microscope. The nearer to the observer the object is the larger it appears. Since the human eye does not easily focus on an object that is closer than 10 inches from the eye it is necessary to place a lens between the eye and the object to assist in accommodation. This lens also possesses a certain magnification. The total magnification of a compound microscope is the product of the magnification of the objective and the eyepiece. Thus the oil emersion objective having a magnification of 98 when used with eyepiece 10X having a magnification of 10 gives a total magnification of 980.

The most important property of the optical system, however, is not magnification but resolving power. The ability to see fine detail in the object is called resolving power. Without resolving power it is impossible to make a true interpretation of the object. A quantity called the numerical aperture is the measure of the resolving power of a microscope objective. The numerical aperture is expressed by the equation N.A. $= n \,.\, \sin a$ where n is the lowest index of refraction between the object and the objective, and a is the angle between the extreme ray of the axial bundle and the axis. This angle cannot be much more than 60°. For this angle the numerical aperture would be 0.86 if the lowest medium between the object and the objective were air having an index of refraction of 1. Oil having an index of refraction of 1.52 would give a numerical aperture of 1.30 providing the condenser has the same numerical aperture and the oil is placed between the condenser and the slide. A thorough understanding of resolving power however depends upon an understanding of the theory of diffraction. Light is a wave motion and when the rays forming a wide angle cone impinge on an object, they are divided by a process called diffraction into groups with a separation between them. The light is fanned out into a wider cone of larger angle and the objective must take in all of these groups in order to show all the detail of the object. The short wave lengths of light are jostled less than are the longer wave lengths and the wave length of light is changed when it enters a medium of different index of refraction; that is, it is reduced in the ratio of the index. Consequently a high index medium between the object and the objective will increase the resolving power of the objective.

The limits of microscopical resolution are now placed at objects which measure not less than 0.25 or 0.2 micron in diameter. With regard to bodies smaller than this we have laid great stress upon permeability as a criterion for size. These we have called filter-passers. Some of these filter-passers we have been able to see by making use of the dark-field microscope and the ultramicroscope.

In the discussion of the optics of the compound microscope given above only bright-field microscopy has been considered.

That is, the entire field of view is lighted and the objects appear as colored pictures, or as shadows, and in some cases as silhouettes on a white ground. In dark-field microscopy the field is dark and the objects appear as if they themselves emit the light by which they are made visible. Dark-field microscopy deals with relatively large objects of 0.2 micron or more in diameter or, in other words, objects that come within the resolving power of the microscope. Ultramicroscopy deals with objects so small that they do not show as objects with detail but their presence is inferred by points of light. An analogy is found in the Tyndall phenomenon in which particles of dust appear as points of light in a sunbeam passing through a darkened room. These two types of microscopy are said to merge as pointed out by Gage(2) who states;

> Because in studying objects like saliva, etc., with the microscope designed especially for dark-field work, some of the objects seen will show details, but some are so small that they show simply as points of light usually in the form of so-called diffraction discs. The larger objects in the saliva come in the province of dark-field microscopy, and the smallest ones, of ultra-microscopy, and in this case the instrument used might with equal propriety be called a dark-field or an ultra-microscope.

In dark-field microscopy objects are illuminated from above the stage by direct light or from below the stage by transmitted light. When the light is from above the stage the background must be nonreflecting. The background is obtained by placing the object on a glass slide on black velvet. When transmitted light is employed the objects must be able to deflect the light into the microscope. The light from its source must not be allowed to pass directly into the microscope. Objects deflect light into the microscope by reflection, by refraction, and by diffraction. The efficiency of the dark-field has been increased by such inventions as the paraboloid illuminator.

With the dark-field certain bodies have been made visible in some detail which are either only faintly perceptible or not at all visible with the ordinary compound microscope. Such for example are certain spirochætes.

Many diseases are caused by filterable viruses, which for the most part are invisible. Attempts have been made to calculate the probable size of these agents. D'Herelle believes that the diameter of the bacteriophage particle is 20 or 30 millimicrons, and believes that he has actually seen these particles with the ultramicroscope. Duggar and Karrer consider that the virus of tobacco mosaic is approximately the same size as particles of hæmoglobin, or about 30 millimicrons. Bechhold states that a mass of fifty molecules of egg albumen measures between 4 and 10 millimicrons. In actual use, the limit of microscopic observation with direct illumination is about 0.00025 millimeter, with oblique illumination by means of ultra-violet rays, and with the aid of a monobromated naphthalene immersion 0.00012 millimeter. Everything below this is called ultramicroscopic.

Many bacteria approach the limits of microscopic perception yet they have been discovered and studied with the ordinary microscope without dark-field illumination. The bacillus of influenza measures only about 0.0012 millimeter long and 0.0004 millimeter thick; the bacterium *Spirillum parvum* has a diameter of 0.0001 to 0.0003 millimeter; while *Micrococcus indigofera* is said to be only 0.00015 thick. Manifestly none of these should be regarded as an ultramicroscopic virus although it may be filterable in certain stages.

In regard to the ultramicroscope Damm(3) in Prometheus states:

> The ultramicroscope enables us to penetrate a long way into the domain of the invisible. According to Siedentopf, particles may be perceived with the ultramicroscope which have a diameter of about 4/1,000,000 to 6/1,000,000 millimeter. These though, are magnitudes that approach very closely to the molecular dimensions of complicated organic compounds, in some cases even attain them. According to O. E. Meyer the molecule of hydrogen has a hypothetical diameter of 1/10,000,000 millimeter; according to Jaeger, the molecule of ethyl-alcohol has a diameter of 5/10,000,000 millimeter, the molecule of chloroform a diameter of 8/10,000,000; according to Lobry de Bruyn, the diameter of the molecule of starch is 5/1,000,000 millimeter. Consequently the molecule of starch must be within the reach of ultramicroscopic perception.
>
> Thus, the investigator has prospectively before him, in dependence on the contingent increase of the intensity of light, the ability to see in the dark-field of the ultramicroscope those theoretically disclosed particles, the molecules, which seemed beyond the reach of our sight forever, and the hope of following with his eyes the play of their attractive and repellant forces. Thus, everything depends on the enhancement of the illumination. Yet the brightness of the ultramicroscopic particles begins to decline with the sixth power of the diameter. Should it prove to be possible with the aid of the ultramicroscope to obtain a deeper insight into the form and structure of matter, a positive service will be done to philosophy.

The ultramicroscope, similar to the phenomenon of Tyndall, is based upon the effect of contrast. The particles to be investigated must be under a very strong illumination and this illumination must be that of the dark-field. The ultramicroscope does not give a reproduction in the exact microscopic sense, but merely a proof of existing small particles which have an index of refraction differing from that of the surrounding medium.

It lacks reproductive power. The ultramicroscope then may be regarded only as an ordinary microscope to which the dark-field principle with the strongest possible light is added.

The ultramicroscope was invented in 1903 though the principle of contrast has long been known. It is surprising that so little progress has been made in the development of the ultramicroscope during the last quarter of a century. It remains practically where Zsigmondy left it. Zsigmondy was interested in a study of ruby glass—a deep red but transparent variety which is made by melting together glass and metallic gold. When a sunbeam, brought to a focus by a convex lens, is thrown upon a block of ruby glass the converging pencil of light within the glass becomes luminous and visible like a sunbeam traversing cloudy water or air filled with dust. Faraday thought this indicated that ruby glass owes its color to extremely small particles of gold scattered throughout its subtance. The dimensions of these particles must be less than the Helmholtz-Abbé limit of visibility. In 1906 Michaelis(4) stated:

> The experiments of Fizeau and Ambronn, however, had proved that under conditions of illumination different from those which are customary in microscopic observations the light which emanates from very much smaller objects can be perceived. For example, when a silvered glass mirror is held between the eye and the sun it is possible to detect scratches in the coating the width of which is much smaller than the assumed limit. And against the dark background of the sky stars are seen whose diameters are so small that they appear as points of light in the most powerful telescopes. In these cases what is seen is not the "counterfeit presentment" of the object but merely an impression of light. In other words, we can see luminous objects of almost infinite smallness, in the sense of perceiving their light, if they are very bright and have a dark background.—Zsigmondy reasoned that if particles of gold in ruby glass could be made luminous they would become visible in this restricted sense.—Zsigmondy, accordingly, illuminated a block of ruby glass with a converging pencil of light and examined it with a microscope the tube of which was perpendicular to the pencil. He found that a comparatively low power sufficed to show the separate particles of gold, shining in the dark field like countless stars.

Later a practical instrument was made in the form of the ultramicroscope by Karl Siedentopf in Jena.

The chemical application of the ultramicroscope has been most interesting in the field of colloids. Solutions such as albumen, glycogen, colloidal metals, and many dye stuffs appear transparent when seen by transmitted light unless they are deeply colored, but a cone of light projected into the solution by a lens is brightly luminous and the light is found to be polarized. Particles of colloidal gold are found to dance to and fro and in straight and zig-zag lines. A colloidal solution of gold appears red by transmitted light, but the luminous particles appear green in the ultramicroscope. The size of such particles in colloidal solution have been determined as ranging from 6 to 40 millimicrons.

The hope that the ultramicroscope would demonstrate the ultraviruses has not been realized. Since the ultramicroscope does not give the form of an object it is difficult to discern the difference between living viruses and particles of albumen or other substances for the latter are also endowed with rapid independent motion in colloidal solution.

In 1925 Barnard(5) attempted to apply the principles of the ultramicroscope with ultra-violet light photography. This author believes he has been able actually to photograph ultraviruses by this method. Only future experimental work will determine if this is so or if the bodies photographed by him are of the nature of spherical bodies commonly found in most organic fluids. The lower limit in size for living things is unknown. There appears to be evidence that the ultraviruses are living, but it is not certain that all of the ultraviruses will eventually be classed as animate. Zinsser and Tang(6) have attempted to arrive at the relative size of several different substances by filtration at a known P_H through graded filters prepared of collodion. The results of their experiments show an order of magnitude of the various substances tested as follows: Crystallized egg albumen; crystallized serum albumin; trypsin; collargol; casein; bacteriophage, Rous sarcoma, and herpes virus; and arsenic trisulphide. Levaditi and Nicolau(7) and Levaditi, Nicolau, and Galloway(8) found that the virus of foot-and-mouth disease is filterable through membranes which hold back trypsin. While such experiments give only the relative size of different substances and viruses they also indicate that the viruses closely approximate the same size as a group.

FILTRATION AND FILTERS

We have seen in previous chapters that filtration is a relative concept and that it does not represent a sharp dividing line. The one constant thread of evidence upon which we have based a classification of viruses has been the question of their filterability. There are many objections to the use of filters, but their use has been convenient in the study of diseases of unknown etiology. These objections have been discussed elsewhere, but they are of sufficient importance to bear repetition at this point.

There are many different kinds of filters, and none is free from objection. Porous candles have been prepared with a variety of substances; such as, porcelain, infusorial earth, asbestos, plaster of Paris, calcium carbonate, and magnesium oxide. Physical chemists have found such filters objectionable for the study of colloids because of their great absorptive properties. While most of the ultraviruses will pass filters which retain ordinary bacteria, a number of factors make their use unsatisfactory. No ultravirus has been obtained in pure state; even the granules of vaccinia are not to be regarded as pure virus. The technical habits of the investigator in the use of these filters are of great importance when results are to be analyzed and evaluated. The controversy that has been waged in connection with the filterability of rabies and vaccinia virus is a case in point. Some investigators have described these viruses as filterable, while others have either not succeeded at all in filtering them or in only rare instances have succeeded, in which case the integrity of the filter employed has always been questioned. Other factors such as type and amount of pressure employed, duration of the filtration, the dilution of the material filtered, its viscosity, the amount of solid matter present, the reaction of the material, the possible variation in size of the virus at the time of filtration, type of filter employed, the use of control organisms to control such filters, are all important considerations when results are to be interpreted. Furthermore, we now know that the electric charge carried by the filter and that carried by the virus are also important considerations. Recently Kramer(9) has shown that by preparing a filter of calcium carbonate and magnesium oxide of positive electrical charge bacteria, viruses, and colloids used in his tests may be witheld though these agents readily pass through filters made of siliceous material carrying a negative charge.

It seems appropriate to describe briefly the proper use and the limitations of some of these filters which are in daily use in most laboratories. Probably the three filters in most common use are the Berkefeld filter; the Chamberland-Pasteur filter; and the Seitz-Werke asbestos filter. In some laboratories Doulton filters are employed.

The Berkefeld filter.—The Berkefeld filter is obtainable in several grades of fineness upon which depends the rapidity of filtration. This filter is manufactured in three grades; the W, which is very fine or slow filtering; the N, which is a normal or medium filter; and the V, which is a coarse or rapid filter. For most work the N filter is employed. These filters are obtained in different sizes ranging from 10 by 2 inches to 2 by 1 inch. There are three other sizes on the market which fall between these two extremes. For very small quantities the smallest size suffices. In the process of manufacture filters even of the same grade differ slightly from each other, but for ordinary purposes they may be considered uniform. A new filter before it is used should be cleaned carefully, after clear water has been filtered through it, it should be placed in cold water and boiled. The filter candle and all attachments must be carefully sterilized before use. The usual custom is after thorough cleansing, to assemble the filter ready for use, to wrap it in paper, and sterilize it in the autoclave. The filtering flask can be sterilized by hot air or by autoclave, the former method is usually employed. Just before filtration the assembled filter and flask are put together. Customs vary in different laboratories in the preparation of these filters. The entire set-up may be sterilized in the autoclave ready for use, but we have found this procedure unsatisfactory for general use. After use the filter candle should be freed from all coagulated matter by running large quantities of water through it. In some laboratories a weak permanganate solution is then passed through the filter followed by a weak carbonate solution but this procedure is unnecessary. The filter is then boiled and its surface should be lightly scrubbed with a fine brush. Such filters may be employed for further operations, but in time the candles become clogged. Careful heating to glowing in an oven will renew them to some degree but frequently this causes fissures, and filters so treated should be carefully tested before further use. In all cases these filters should be tested before use with a broth culture of some bacteria such as *Bacillus prodigiosus*. If the liquid to be filtered contains a large amount of solid matter it should first be filtered through paper or paper pulp. Sand may also be used for this purpose. Slight suction may be used to hasten filtration. When filtering through the filter candle suction or force pressure is employed. Suction is obtained by means of a filter pump which is attached to a water faucet, and pressure is obtained by air under pressure. For this purpose a hand pump may be used with a cylinder to equalize the pressure. A manometer records the pressure used. The amount of suction or pressure employed is very important. Many organisms decidedly non-filterable may be driven through a filter if the pressure is too great. The Mandler diatomaceous filters are tested for certain pressures. These vary from four to six pounds air pressure. Filters of this type are in common usage in most laboratories. Very large filters employed in the preparation of biologic products for commercial use will withstand very high pressures. By various arrangements such as the Hill apparatus the ordinary pressure of city water services of 40 pounds per square inch may be increased to as much as 300 pounds per square inch.

Duration of the filtration process has already been mentioned. Filtration should proceed with rapidity commensurate with the efficiency of the filter. It is possible for bacteria literally to grow through the filter if sufficient time is allowed. This, of course, should be avoided. The entire process of filtration should be carried out in a manner which will preclude contamination of the filtrate. In spite of every precaution contaminations will occasionally occur. Before removing the filtrate from the filtering flask when suction is employed a short period should be allowed for the equalization of pressure as a sudden release of the vacuum may result in the entrance of contaminated air. For most purposes the Berkefeld or the Mandler filters will suffice though some investigators prefer to work with other types of filters, in some of which the technic for use is slightly different from that given above.

The Chamberland-Pasteur filter.—These filters are made of unglazed porcelain. Various grades of fineness are obtainable. For ordinary purposes grade F is employed; grade B is suitable only for pressure. These filter cylinders are made in many different sizes, the largest are used for filtering large volumes. Special apparatus has been devised equipped with filter, manometer, pressure pump, etc. for the filtration of serums and toxins in large quantities. For general laboratory use the smaller filter cylinders are very satisfactory. Filtration with these cylinders is usually carried on from within-out instead of without-in as in the case of the Berkefeld and Mandler filters. However, the latter are also manufactured for use in this manner. The usual precautions for the use and care of porcelain filters pertain as in the case of the Berkefeld candles.

The Seitz-Werke filter.—The filtering substance of this filter is the asbestos disk. These disks are manufactured in many different sizes for use when different quantities of liquid are to be filtered. The flat asbestos disk is inclosed in a chamber resting upon a screen which is of German silver. The cup is screwed down upon the disk which seals the apparatus around the edge. The entire apparatus is mounted by means of a perforated rubber cork to a filtering flask. It is now ready for sterilization in an autoclave. The filter and flask may be sterilized separately if desired and mounted when ready for use. This type of filter is very economical and efficient. One disadvantage is the initial cost since different sizes are necessary for varying quantities of fluid to be filtered. The apparatus is easily assembled and cleaning is unnecessary since new disk are used for each operation. This apparatus is designed for either small or large quantities of liquid and has become quite popular for general laboratory use.

Ultrafilters.—Colloidal chemists have found the porous filter candles unsatisfactory for work with colloids because of their adsorptive qualities. As a result they have made use of different types of ultrafilters for the study of these solutions. One of the commonest types of ultrafilters in laboratory use is the collodion membrane. Still other types of ultrafilters have been prepared by impregnating filter paper with collodion. This type of filter and one which is obtainable on the market is known as the Columbia ultrafilter. This filter will withstand high pressures.

Ultrafilters prepared with collodion are of essentially two types. They may be prepared as sacs or as flat disk membranes. The great difficulty in the use of these filters has been to obtain uniform membranes. Several different mixtures of collodion have been recommended as well as different technics for preparation and this has not contributed to uniformity. A mixture which has been in common usage in various laboratories consists of:

Alcohol, 96 per cent, 250 cubic centimeters.
Ether, 65 per cent, 250 cubic centimeters.
Nitrate of cotton, 10 grams.

In general the size of the pores in collodion membranes depends upon the amount of alcohol and the amount of nitrate of cotton. By increasing the amount of alcohol and lowering the nitrate of cotton the pores become larger. Different degrees of porosity may be obtained by varying the quantities of the ingredients. It is well when preparing collodion solution to allow the mixture to stand for a week or ten days in the incubator at 37.5° C. before using. Frequently, bubbles form in the membranes as a direct result of temperature, and if the collodion is warmer than the surface of the glass upon which the membrane is formed these will not occur. Futhermore, the incubation of the collodion tends to ripen it and the membranes will be of a tougher consistency than otherwise. After preliminary incubation the solution should be preserved at a low temperature and should be warmed for a short time just before using.

There have been several methods advocated for the preparation of collodion membranes. Membranes may be formed upon the surface of test tubes by dipping the thoroughly cleaned test tube into the solution of collodion thereby coating its surface. Several coats of the collodion may be applied in this manner. It facilitates the removal of the membrane from the test tube if the tube is first prepared by blowing a bulbous enlargement near the center and coating the tube to the center of this enlargement when the membrane is made. This enlargement is produced by heating the test tube in a blast flame at the point desired, rotating the tube meanwhile so as to heat it equally on all sides. At the proper moment, which is easily learned by experience, one blows in the open end of the tube and produces the enlargement to the size desired. This tube serves as a mold, and when coating the mold with collodion it is best to withdraw the mold or tube slowly. After removal of the mold from the collodion the tube is held in a horizontal position, rotated and dried to the proper consistency. This is determined by experience. It is important for the drying to proceed just to the proper degree otherwise the membrane may easily tear when removed from the tube or if dried too long the film may wrinkle when removed from the mold. Several coats of collodion may be given in the above manner depending upon the thickness of the film desired.

When the final coat is given and the drying operation is completed, the tube is placed in cold water for a few seconds, then is separated from the tube at the top and under running water the film or sac is removed by turning it back upon itself. The sac is then placed in distilled water or it may be preserved in a 20 per cent solution of alcohol. The sacs when used are attached to one end of a glass tube of the same size as used for a mold but open at both ends. The sac may be attached to the tube with a ligature which can be covered later with fresh collodion which will form a perfect seal at the joint. The sac should then be filled with distilled water and be kept immersed in distilled water until used.

Collodion sacs of this type may be made in many different sizes depending upon the size of the mold employed. For very large sacs the coating with collodion should be very heavy in order to increase the toughness of the membrane. The preparation of such membranes is largely a matter of practice and with experience one's proficiency increases rapidly.

Collodion membranes are also made as flat disk films. These are prepared by pouring the collodion upon round plates of glass. The glass plates should be perfectly clean, dry, and smooth and should rest upon an absolutely level surface. With a given solution of collodion and a known size of glass plate the amount of collodion to be used can be determined by experience so that each film of a given size will be uniform. As in the case with sac-films the drying procedure is very important, and it is well to time this operation according to the temperature of the atmosphere so that uniform films are obtained. This is chiefly a matter of experience and practice. The disk-films are removed under water as are the sacfilms and are then preserved in 20 per cent alcohol for future use. In some laboratories it is the custom to pass these films through several strengths of alcohol before reaching the preservation strength indicated. An apparatus similar to that employed for the asbestos disks of the Seitz-Werke filter can be used for the disk films prepared with collodion.

For work with ultraviruses it is necessary to employ sterile films. It is difficult to sterilize collodion membranes without altering their permeability. It is possible however to sterilize these films provided they are first passed through a process of denitrification. This is accomplished by filling and immersing the sac or diskfilm in a 25 per cent solution of ammonium hydrosulphide and heating in a water bath at 50° C. for forty-five minutes. The film or sac is then washed with a 10 per cent solution of ammonia and finally with distilled water. The sac is then thoroughly dried and inflated with air under constant pressure to prevent wrinkling. Denitrification also reduces the thickness of the membrane somewhat, and consequently the adsorptive property of the membrane, and increases the toughness of the film.

According to d'Herelle(10) a satisfactory method for sterilizing collodion films after denitrification is to fill the sac with 80 per cent alcohol and immerse it in the same solution. This is then placed in an autoclave, the water of the autoclave being replaced with 90 per cent alcohol. He states:

> When all of the air has been drawn off (this is shown by the fact that the alcohol will commence to distill over and with cooling will condense drop by drop in the condenser) close the exhaust valve, and regulate the source of heat in such a way that the autoclave will have a pressure of 1/5 to 1/4 of a kilogram per square centimeters. Maintain this pressure for 30 minutes, allowing for the autoclave to remain closed until after cooling is completed.

According to this author this method of sterilization is equally effective whether denitrification is carried out or not, and except for particular investigations this method renders denitrification unnecessary. After sterilization the alcohol in the sac is removed and is replaced by sterile distilled water for several minutes. When this is removed the ultrafilter is ready for use.

Other methods have been devised for preparing collodion sacs and the method of choice is largely a personal one. Novy recommends the preparation of sacs upon test-tube molds as described above, but in addition he makes a small hole in the bottom of the tube through which water can be forced by blowing, and the removal of the film is facilitated by this procedure. Still another method is to coat the inside of the mold, whether test tube or flask, with collodion and such films are also readily removable.

At best the ultrafilters are not free from objection. In the hands of different workers these films are not prepared according to any standard method and with standard reagents. As a consequence films vary greatly in porosity. It is difficult then to correlate the results obtained by different investigators. In general they are satisfactory for ordinary use but there is much room for improvement.

Animal membranes may also be used as filters. For the most part these have been used in vivo. It is apparent that animal membranes cannot be preserved for any length of time outside the body. We have attempted filtration of a substance such as the bacteriophage through the normal barriers of the central nervous system in rabbits. Grasset(11) injected gravid rabbits and guinea pigs with bacteriophage and demonstrated that the bacteriophage does not pass through the placenta to the fœtal blood. Bacteriophage injected intraspinously into rabbits is recovered from the blood stream, though not vice versa.

CULTIVATION OF FILTERABLE VIRUSES

There is abundant evidence that several of the filterable viruses or ultraviruses have been cultivated outside the animal body; that is, they either survive or multiply for a time in artificial media. The viruses of pleuropneumonia in cattle and of agalactia contagiosa in sheep and goats are both visible and filterable. Both of these viruses have been cultivated in vitro. We believe there should be no objection to the term "in vitro" regardless of the constituents of the medium employed provided the interpretation of this term is "outside the animal body." Both of the viruses mentioned above then have been cultivated in vitro. Likewise this term should with propriety be used when fresh animal tissues are added to the culture medium. In the cases of pleuropneumonia and agalactia the viruses have been cultivated in Martin's bouillon to which serum is added. Beef and rabbit serums are satisfactory for the virus of pleuropneumonia, and serums from rabbits, sheep, goats, horses, cattle, and man have all been found to be satisfactory in the case of agalactia contagiosa virus according to Bridré and Donatien.(12) Furthermore, both of these viruses have been cultivated upon solid serum-agar media. The addition of lactose and mannite also favor the growth of the virus of agalactia contagiosa.

There is little doubt that the vaccine virus and the herpes virus have remained viable in vitro in mediums containing fresh animal tissues. It is true that the propagation of these viruses has been carried only to a certain .point, but there seems to be some evidence that actual multiplication has taken place. The agent producing the Rous sarcoma, if a virus, may also be said to have been cultivated in the presence of living cells. There is some evidence that these chicken tumors are produced by minute tumor cells which are capable of passing filters. If this view should prove to be correct it would not be proper to consider the cultivation of this agent as the cultivation of a virus but rather as the cultivation of the actual tumor cells themselves. Flexner and Noguchi(13) have described the cultivation of the virus of poliomyelitis in a medium consisting of human ascitic fluid to which was added a fragment of fresh rabbit tissue. Levaditi(14) has stated that the virus of poliomyelitis survived or multiplied in fresh spinal ganglia of monkeys when this tissue was placed in plasma. There still remains a doubt, however, that the "globoid bodies" are etiologically associated with poliomyelitis. This virus is also said to have been cultivated upon a solid medium consisting of agar, ascitic fluid, and a fragment of sterile tissue.

It is well known that vaccine virus may be propagated through inoculation of the cornea of rabbits, and Guarnieri bodies may be demonstrated in the lesions. Harde(15) found that this virus can be propagated in the presence of living corneal cells and that the virus does not multiply if these cells are killed by freezing or by hypertonic salt solution. Cracium and Oppenheimer(16) have also described the cultivation of vaccinia "granules" in vitro with embryonic tissues and state that they remain alive for as long as seventy-one days as tested by rabbit corneal inoculation. Cultivation of this virus in the presence of living cells has also been reported by Parker and Nye.(17) Whether or not these experiments have demonstrated multiplication of the vaccine virus is largely a matter of opinion, for it may be pointed out that only survival of the virus may have been demonstrated. However, it appears likely that actual multiplication up to a certain point has been demonstrated. The same statement may be made concerning the cultivation of the herpes virus according to the experiments of Parker and Nye,(18) although actual cultivation in the sense of multiplication seems more doubtful with this virus. All attempts to cultivate this virus by Le Févre and McKinley(19) failed, though a great variety of methods employing fresh tissues in various mediums under different conditions were employed. Furthermore, it is well known that the virus of herpes is active and will produce experimental encephalitis in rabbits even when diluted to 1:100,000 or more. It seems probable that only survival of this virus has been demonstrated in artificial culture media, at least actual multiplication should be accepted with conservatism.

In 1913 Noguchi(20) reported the cultivation of the virus of rabies. These cultures were obtained in ascitic fluid to which was added a small fragment of kidney from a healthy rabbit. The cultivation of this virus has never been confirmed and is doubtful. It is said that Noguchi himself later thought the pleomorphic bodies found by him in his cultures were in reality yeast cells. More recently this author has described the virus of trachoma.(21) On special media Noguchi obtained a Gram-negative bacillus which when injected into monkeys produced a granular conjunctivitis which "had an appearance strikingly like that of the human trachomatous conjunctiva in the early stages of the disease." This work has not been confirmed.

The virus of fowl plague is said by Landsteiner(22) to be adherent to the blood cell and can be cultivated up to the tenth generation by placing defibrinated blood upon agar plates. Landsteiner and Berliner(23) also found that this virus would not multiply if laked or frozen blood was used. These authors believe that this virus does not multiply in the absence of living cells.

Wolbach and Schlesinger(24) have described the cultivation of the virus of typhus fever and of Rocky Mountain spotted fever in a tissue-plasma culture for a length of time corresponding to the life of the endothelial cells multiplying in the cultures. The microörganism can be found in the endothelial cells in large numbers. Also brain-tissue cultures from typhus guinea pigs have proved successful. Plasma from guinea-pig blood is collected in paraffin-lined tubes, centrifuged and chilled. The tissue for cultivation is cut into blocks and is kept immersed in Ringer's solution, then transferred to a sterile cover slip, and covered with sterile plasma. In this manner the virus may be kept alive from four to six weeks. Noguchi(25) has also been able to cultivate a rickettsia-like organism from the tissues of the tick *Dermacentor andersoni,* the vector of Rocky Mountain spotted fever, but he has not identified these organisms to be the actual virus of this disease. From the experiments of Wolbach and Schlesinger there seems to be no doubt that at least two of the so-called rickettsia viruses are cultivated in vitro in the presence of living cells.

Only two viruses (of pleuropneumonia and of agalactia) that are apparently cultivable in vitro in the absence of living cells have been discussed. Both of these viruses are visible and filterable. All the other viruses (vaccine virus, herpes virus, virus of poliomyelitis, rabies, and fowl plague) are ultraviruses, with the exception of the trachoma virus recently described by Noguchi as a Gram-negative bacillus and the rickettsia viruses of typhus fever and Rocky Mountain spotted fever. The latter two are both visible and are not filterable. Noguchi's trachoma bacillus is visible, but nothing is known regarding its filterability. This microörganism, however, according to Noguchi, does not require living cells in the culture medium to permit its growth. Cultures were obtained on leptospira medium consisting of a semisolid medium containing fresh animal serum and hæmoglobin, or blood agar slants containing certain carbohydrates. All the ultraviruses are invisible and filterable, and they can be cultivated only in the presence of living cells, if at all, and this is doubtful in regard to some of them if actual multiplication is to be considered as the criterion for cultivation. The virus of the Rous sarcomata is a special case since there is some doubt of its being a virus and since there exists some evidence that its propagation is brought about by extremely minute sarcoma cells which are capable of passing through a filter. Whether virus or tumor cell the only evidence advanced for its cultivation in vitro has been in medium containing living tissue cells such as fragments of chick embryo (Gye(26)). According to Nakahara(27) the cells of the Rous chicken sarcoma are often filterable through the coarse and medium Berkefeld filters, and a small number of these cells can be demonstrated in a proportion of the filtrates, and, further, this tumor can be transmitted by an injection of a very small number of washed sarcoma cells. This author concludes that the transmission of the Rous chicken sarcoma may not be independent from the actual transplantation of viable sarcoma cells.

There is still doubt in the minds of many that the bacteriophage is actually a living virus as contended by d'Herelle, and for that reason the subject has not been given great prominence in this review. Certainly all will admit that the lytic principle increases at the expense of the lysed bacterium and in this sense it is cultivable in the presence of living cells. Reports purporting to demonstrate the increase of the lytic principle in a medium containing only dead bacteria have not yet been accepted. Furthermore, in common with the ultraviruses, the bacteriophage increases in a medium containing young living cells.

There are certain other diseases that have been thought to be caused by filterable viruses with regard to the cultivation of the causative agents of which the data are conflicting. Among these may be mentioned yellow fever, influenza, Oroya fever and verruga Peruviana, scarlet fever, measles, German measles, and the common cold.

Yellow fever, it now appears, must be placed with the filterable virus diseases, although it may eventually prove to be one of the rickettsia diseases. At least there now appears to be conclusive evidence that Noguchi's *Leptospira icteroides* is not the cause of the disease. As these lines are being written the news of Noguchi's death from yellow fever in Africa is being flash over the world. It was the hope of all that Noguchi would be able to discover the virus of this disease, and he has given his life in the attempt. The news of his untimely death and sacrifice comes as a shock to the scientific world, and science has lost a great investigator and friend. The true virus of yellow fever remains unknown. Only a short time before his death Noguchi reported *Bartonella bacilliformis* as the infectious agent of Oroya fever and verruga Peruviana and proved that these two conditions are manifestations of one disease. This organism was cultivated by Noguchi on his leptospira medium.

Scarlet fever, measles, and German measles are now thought to be caused by streptococci. The evidence is practically conclusive for scarlet fever, fairly convincing for measles, but doubtful for German measles. It is probable that in time streptococci will be definitely identified as the causative agents in these three diseases. So far strains of streptococci have been cultivated from cases of all three. Rivers(28) states, "Streptococci have been given an etiological rôle in many virus diseases. It is unlikely, however, that they cause all of the following diseases: poliomyelitis, lethargic encephalitis, influenza, measles, and German measles."

Influenza and common colds have both been thought to be caused by filterable viruses. *Bacterium pneumosintes* cultivated by Olitsky and Gates(29) from influenza cases, and the virus obtained from cases of common colds, described by Kruse(30) and Foster,(31) and presumably cultivated by the latter, have not been definitely established as the true etiological agents in these diseases. Foster himself now seems to believe that his culture experiments in the cultivation of the common cold virus were doubtful. However, the writer has been unable to find any publication by Foster in which he has withdrawn the conclusions from his former work in which he stated, "Although conclusive proof of its nature has not been adduced, the experiments suggest that the microörganism described bears a definite relation to the true infective agent of common colds." It seems altogether probable that both of these diseases are caused by filterable viruses, yet adequate proof is lacking.

In 1925 Olitsky(32) reported the successful cultivation of the virus of tobacco and tomato mosaic. For mediums he employed an aqueous extract of the stems, leaves, and shoots from tomato plants which he knew to be free from mosaic. This extract was sterilized by filtering through a Berkefeld N filter. After several days when the medium was known to remain sterile, inocultations were made with the filtered juice from infected plants. The virus remained viable and was capable of inducing the disease in healthy plants after thirty-three days. Subcultures were made after seven to ten days. It is well known that a dilution of 1:1,000,000 of the mosaic virus is practically innocuous to healthy plants. Olitsky failed to infect any of thirty plants tested with this dilution. A dilution of the original virus of 1:1,000,000 was reached by the fourth subculture in Olitsky's experiments. He states that, "The fourth subplants in four experiments and the fifth in three others caused typical mosaic disease in plants previously normal. The seventh, ninth, and tenth subplants induced the affection in three, three, and one tests respectively. More remote subplants in these series were not made." This author states that, "The conclusion seems justified that the incitant of mosaic disease of tobacco and tomatoes is a living microbic body which can be cultivated in an artificial medium." Mulvania(33) and Purdy(34) were both unable to confirm these results, and at present the question remains status quo. This question is fundamental and more attempts should be made along this line. The unquestioned successful cultivation of any one of the ultraviruses will undoubtedly present leads which will result in the cultivation of others.

Young living cells must be present in the artificial mediums employed for the cultivation of certain of the ultraviruses. The bacteriophage increases at the expense of young bacteria. Mosaic disease is manifested only on young leaves. Virus diseases of insects occur only in certain stages of their development, and it is well known that in man and animals virus diseases are manifested chiefly in young healthy individuals. In this connection Rivers states:

> Injury also plays an important rôle in the infectiousness of many virus diseases, not the rôle, however, of furnishing a *nidus* of dead tissue for the growth of the viruses, since dead tissues do not promote their growth, but the rôle of furnishing young cells or growth-promoting factors usually found in their vicinity. Injury necessitates repair. Restoration, as is well known, is accompanied by young cells.

This phenomenon is seen in connection with the Rous sarcoma where more rapidly growing tumors are produced when both virus and kieselguhr are injected together than when virus is injected alone. Furthermore, in this regard, Rivers states:

> More metastases are found in the ovaries during egg laying seasons than during quiescent periods. These phenomena are probably due to the presence of young cells participating in the repair of injured tissues.

It is also well known that viruses have a species specificity as illustrated by the virus of poliomyelitis, which attacks only man and monkeys; by the wilt virus, which attacks only one species of caterpillar, etc. Furthermore, viruses seem to possess to some degree at least a selective tissue localization. Rabies and poliomyelitis viruses attack the nerve tissue, and smallpox and chicken-pox viruses attack the skin. Vaccine virus, herpes virus, and chicken-pox virus, however, attack both exodermal and mesodemal cells. While too much stress should not be given this observation, the fact remains that there is certainly a tendency for such tissue selection. We do not know definitely whether viruses multiply in vivo within the cells or outside the cells. We do know that virus diseases produce profound effects upon the cells. The cells may increase in size and exhibit amitotic division of the nuclei. This has been designated as ballooning degeneration. Later the cells die and go to pieces. Rivers states in this connection:

> Two forces seem to operate: one stimulating the cell, the other destroying it. Consequently the picture produced by virus disease is more or less dependent upon which of these forces predominates. Chicken-pox, foot-and-mouth disease, zoster, variola, and lysis of bacteria by phage are diseases in which destructive agencies predominate. Rous sarcoma, contagious epithelioma, fowl leukemia, and warts are diseases in which stimulating forces are dominant.

In the latter class may also be mentioned epithelioma of fishes and molluscum contagiosum in man. It is probable that this effect upon cells may be related to the production of the so-called inclusion bodies but as yet we have no proof of this theory.

THE NATURE OF VIRUSES

There is abundant evidence to show that at least most of the viruses associated with disease processes are endowed with life. We cannot state definitely that all viruses exist in a corpuscular state or that a "liquid fluidum vivum" is beyond the realm of possibility. Likewise we know not if certain viruses may not fall into the category of a "contagium inanimatum" as suggested by Simon. It is useless to pretend that we know the true nature of viruses and consequently no further discussion is necessary. When the true nature of these agents is known it will be largely through knowledge of their properties. Of these more is known. It is known for example, that viruses have a tendency to be adsorbed by many different substances; that they are in general resistant to physical agents such as alcohol, acetone, chloroform, toluol, formalin, and phenol, and will withstand the action of some of these reagents for varying periods of time depending upon the strenght of the reagents employed and the degree of resistance possessed by the individual virus tested. It has been impossible to demonstrate that viruses respire although this may be due to the limitations of the methods employed and the possibility that oxygen is consumed and carbon dioxide given off in quantities too small to be measured by our crude methods. There is some evidence of mutation in the virus family as there is with bacteria. According to Ashburn, Vedder, and Gentry (35) the virus of smallpox is modified when passed to calves. These authors state:

> While touching the relationship of animal poxes we may here mention, on the authority of Doctor Ruediger, in charge of the serum laboratory,

Bureau of Science, Manila, that the strain of vaccine virus now used by that Bureau and by the Government of the Philippine Islands, a strain with which almost 7,000,000 vaccinations have been made with great success, was derived from a fatal case of human smallpox in 1908, having been passed first through monkeys, and from the second monkey to a heifer, and from the fifth heifer to man. When such a virus is taken from man, passed to monkeys and then to cattle and brought back to man, a localized, noncontagious, mild disease is produced. Having by passage, once lost its power to produce smallpox, the virus *never regains* it, even though passed from person to person (proper hosts for variola virus) for thirty-five or one hundred years.

These authors suggest that:

> Smallpox is due to a dual and divisible virus, one part of which causes vaccinia and the specific smallpox eruption, the other part being necessary for the production of the contagious, generalized, mortal disease with a distinct preëruptive stage and initial rashes.

There seems to be abundant proof in this one example that a virus may be changed when passed from one host to another, and the word "mutation" to designate this change seems to be as satisfactory as any other which might be suggested. Similar observations have been made with other viruses, such as the virus of contagious epithelioma of chickens and pigeons and the virus of the mosaic disease of tobacco and of cucumbers.

It is also known that certain viruses are endowed with other properties which gives some insight into their nature. This is particularly true of viruses that seem to be related, in so far as disease manifestations are concerned, with other viruses. Some viruses for example appear to be epitheliotropic while others are neurotropic. It is well known that such similarities between viruses have in the past confused investigators. Smallpox and varicella are no longer regarded as related, though there still is believed to be some relation between zoster and varicella. Many strains of herpes have been obtained from cases of herpes simplex and from herpes genitalis, but as yet no virus has been obtained from herpes zoster. These similarities cannot be depended upon in gaining an insight into a virus whose manifestations are found to be similar but of which we know nothing except by comparison with some known virus of like properties. Yet all of these properties must be taken into consideration and a given virus may be found to be similar to other viruses in certain respects and different in others. Such information is, of course, valuable.

Intracellular inclusions.—A great deal has been written about the so-called inclusion bodies. This subject has been discussed in detail in Chapter XVII. It must be concluded that the nature of these bodies and their relation to virus diseases is unknown.

BIOCHEMICAL AND PHYSICOCHEMICAL PROPERTIES OF VIRUSES

Of the biochemical and physicochemical properties of viruses little is known. Until it is possible to obtain virus in the pure state it will be impossible to progress far along these lines of investigation. Beyond determining the thermal death point we have been able to do little. The tendency of viruses to be adsorbed by many different substances has already been referred to. Data regarding the functional (enzymatic) activity of viruses are exceedingly scarce. There is no direct evidence that viruses elaborate toxins or other poisons. Recently Olitsky and Boëz (36) have demonstrated by cataphoresis experiments that the virus of foot-and-mouth disease carries an electropositive charge and that its isoelectric range is about P_H 8. These are the sort of fundamental studies that are much needed in this field. A study of viruses along the general lines of colloidal and physical chemistry will undoubtedly add much to the knowledge concerning these agents.

EPIDEMIOLOGY, PREVENTION, AND IMMUNITY

Epidemiologically virus diseases present a wide range of differences. Some are markedly contagious, others are not at all contagious. Some virus diseases are spread by direct contact, others are disseminated only through the agency of an insect vector. Some attack principally certain age groups. Others exhibit definite seasonal variation, regional distribution, and host susceptibility. In general these variations pertain to other types of infectious processes also so that there is nothing peculiar in this as far as the virus disease are concerned. In general one attack of a virus disease, if recovered from, produces a lasting immunity. Immunity to virus diseases is both natural and acquired as is the case with other infections. There are, of course, examples of bacterial infections where one attack of the disease produces a lasting immunity so this is not peculiar to virus diseases. Furthermore, it is known that certain virus diseases do not always protect against subsequent attacks of the same disease. It is dangerous to generalize on this point, but it may with confidence be accepted that there is perhaps a greater tendency among virus diseases to produce a lasting immunity than there is among other infectious processes. The reason for this is not understood. It has been suggested that the virus remains latent in the tissues, viable and capable of continued initiation of immunity but there is no evidence that this is true.

Animals immunized with active virus produce serums which exhibit virucidal properties for the virus in vitro providing the animal so immunized is susceptible to the virus employed. This is true for both the herpes and the rabies virus in rabbits. Furthermore, these animals are protected from subsequent infection. Philippine monkeys, on the other hand have been found to be resistant to the herpes virus when injected intracerebrally with large quantities of virus. The serums from these monkeys exert no virucidal effect upon the herpes virus in vitro. Rabbits are naturally immune to the virus of rinderpest and repeated injections of large quantities of rinderpest infected carabao blood intravenously into these animals do not produce serums which exhibit the slightest virucidal activity against this virus in vitro.

There is evidence which points to a tissue immunity in virus diseases rather than a humoral one though no one will question the fact that evidences of this immunity are frequently found in the serum in the form of virucidal properties, precipitins, and complement-fixing antibodies. In this connection it is also interesting to note that for several virus diseases artificial immunity is produced only by tissue vaccines. In a recent paper we have called attention to Kelser's chloroform-treated vaccine for rinderpest.(37) Immunity against this disease cannot be produced in cattle with chloroform-treated infected blood but is readily produced with other infected tissues treated with this reagent. Rabies vaccine is another case in point while in smallpox no one will be willing to claim as yet that immunity has been produced with pure vaccinia virus. One of the outstanding facts of virus immunity is the fact that practically without exception artificial immunity can be produced only with living virus. In this respect virus immunity differs from bacterial immunity.

Prevention in certain diseases has been well established. No one will doubt the great results obtained with vaccination against smallpox. Unfortunately it is not possible to prevent by this means many of the other virus diseases. In fact for the great majority of virus diseases we must rely solely upon sanitation and hygiene. Such measures as quarantine, isolation of susceptibles, eradication of insect vectors, and destruction of reservoirs of infection aid materially in the prevention of these diseases but these are not permanent.

CONCLUSION

The study of virus diseases and their causative agents is rapidly becoming a specialty and this is well since the combined attention of many investigators is essential to the solving of the many problems which have been presented. Diseases such as influenza, the common cold, poliomyelitis, encephalitis lethargica, and yellow fever are among the greatest problems in medicine to-day. Malignant diseases take their annual toll of human lives in increasing numbers, and the solution of the problem may rest in this field. The economic loss from virus diseases in animals and plants is enormous to say nothing of the lost of time in human labor. The problems call for the concerted effort of the physician, the veterinarian, the plant pathologist, the entomologist, and allied scientists. Only through concerted attention, coöperation, and freely exchanged constructive criticism will any great progress be made. No one can prophecy where future discoveries in the virus field may lead. One of the greatest problems, if not the most important of all, for future investigation in this field is the cultivation of viruses. Not until bacteria were isolated in pure culture was much understood concerning them. Following their isolation in pure culture knowledge of their nature, their chemical composition, their properties, functional activity, and mechanism of infection and immunity became available. It may logically be expected that the same will be true of the ultraviruses. Until they are reproduced artificially in the test tube knowledge concerning them will come chiefly from theory and inference, neither of of which can be depended upon in the absence of fundamental scientific data.

BIBLIOGRAPHY

1. COTTON and MOUTON, Scientific Am. Suppl. 68 (1909) 162.
2. GAGE, Trans. Am. Microscop. Soc. 39 (1920) 95.
3. DAMM, quoted in Scientific Am. Suppl. 80 (1915) 211.
4. MICHAELIS, Scientific Am. Suppl. 62 (1906) 25577.
5. BARNARD, Lancet 2 (1925) 117.
6. ZINSSER and TANG, Journ. Exp. Med. 46 (1927) 357.
7. LEVADITI and NICOLOU, C. R. Acad. 176 (1923) 717.
8. LEVADITI, NICOLOU, and GALLOWAY, C. R. Acad. 187 (1926) 247.
9. KRAMER, Journ. Infect. Dis. 40 (1927) 343.
10. D'HERELLE, The Bacteriophage and Its Behavior. Williams and Wilkins Co., Baltimore (1920).
11. GRASSET, C. R. de la Soc. de Biol. 96 (1927) 839.
12. BRIDRÉ and DONATIEN, Ann. l'Inst. Pasteur 39 (1925) 925.
13. FLEXNER and NOGUCHI, Journ. Am. Med. Assoc. 60 (1913) 362. Journ. Exp. Med. 18 (1913) 461.

14. Levaditi, Compt. rend. Soc. biol. 74 (1913) 1179; 75: 202.
15. Harde, Compt. rend. Soc. biol. 78 (1915) 545. Anns. l'Inst. Pasteur 30 (1916) 299.
16. Cracium and Oppenheimer, Journ. Exp. Med. 43 (1920) 815.
17. Parker and Nye, Am. Journ. Path. 1 (1925) 325.
18. Parker and Nye, Am. Journ. Path. 1 (1925) 337.
19. Le Fèvre and McKinley: (1925), quoted by McKinley in Journ. Infect. Dis. 39 (1926) 441.
20. Noguchi, Journ. Exp. Med. 17 (1913) 29.
21. Noguchi, Journ. Am. Med. Assoc. 89 (1927) 739.
22. Landsteiner, Centralbl. f. Bakt. 38 (1906) 540.
23. Landsteiner and Berliner, Centralbl. f. Bakt., Abt. 1, Orig. 67 (1913) 165.
24. Wolbach and Schlesinger, Journ. Med. Research 44 (1923) 231.
25. Noguchi, Journ. Exp. Med. 43 (1926) 515.
26. Gye, Lancet 2 (1925) 109; Brit. Med. Journ. 2 (1925) 291.
27. Nakahara, Sc. Rep. Govt. Inst. Infect. Dis. Tokyo 5 (1926) 287.
28. Rivers, Journ. Bact. 14 (1927) 217.
29. Olitsky and Gates, Journ. Am. Med. Assoc. 74 (1920) 1497; 33 (1921) 125, 361, 373; 34 (1921) 1; 35 (1922) 1, 553.
30. Kruse, München med. Wchnschr. 61 (1914) 1547.
31. Foster, Journ. Am. Med. Assoc. 66 (1916) 1180. Journ. Infect. Dis. 21 (1917) 451.
32. Olitsky, Journ. Exp. Med. 41 (1925) 129.
33. Mulvania, Science 62 (1925) 37.
34. Purdy, Bot. Gaz. 81 (1926) 210.
35. Ashburn, Vedder, and Gentry, Philip. Journ. Sci. 8 § B (1913) 17.
36. Olitsky and Boëz, Journ. Exp. Med. 45 (1927) 673, 685.
37. McKinley, Proc. Exp. Biol. and Med. (1928). (In press.)

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DENGUE

ITS HISTORY, EPIDEMIOLOGY, MECHANISM OF TRANSMISSION, ETIOLOGY, CLINICAL MANIFESTATIONS, IMMUNITY, AND PREVENTION

BY

J. F. SILER, MILTON W. HALL, AND
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